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TABLE OF CONTENTS

An electron microscope study of mutation in tobacco-mosaic virus. WILLIAM N. TAKAHASHI and T. E. RAWLINS 73

Mild rusty mottle of sweet cherry (Prunus avium). S. M. ZELLER and J. A. MILBRATH 77

Field methods of testing for root-knot infestation. A. L. SMITH and A. L. TAYLOR 85

Factors affecting infectivity, spread, and persistence of Piricularia oryzae Cav. AXEL L. ANDERSEN, B. W. HENRY, and E. C. TULLIS 94

Soybean diseases in Ontario and effectiveness of seed treatment. A. A. HILDEBRAND and L. W. KOCH 111

Organic compounds for control of tobacco blue mold. T. W. GRAHAM, E. E. CLAYTON, J. G. GAINES, T. E. SMITH, and F. A. TODD 125

Stability of labile viruses in desiccated tissue ..... H. H. MCKINNEY 139

Phytopathological Notes 143

Cuticle cracks in tomato fruits ..... P. A. YOUNG

Rusty spot of peach ..... EARLE C. BLODGETT

PHYTOPATHOLOGY

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AN ELECTRON MICROSCOPE STUDY OF MUTATION IN TOBACCO-MOSAIC VIRUS

WILLIAM N. TAKAHASHI AND T. E. RAWLINS

(Accepted for publication September 30, 1946)

Common tobacco-mosaic virus (Johnson's tobacco-mosaic virus 1) obtained from infective plant juice by differential centrifugation has a characteristic particle length of around 300 mμ (6, 8). Stanley (7) and Knight have made a number of contributions showing that at least certain virus mutations are accompanied by a change in the amino acid content of the virus. Melchers and co-workers (5) reported a strain of tobacco mosaic having a mean particle length of 137.5 mμ and another strain having a mean length of 187.5 mμ.

These reported differences between the particle length of common tobacco-mosaic virus and viruses which may be mutants of this virus bring up the possibility that certain mutations may be caused by fracture of the nucleoprotein virus particle. In an attempt to throw light on this question, we have used a virus that is presumably the result of a single mutation of common tobacco-mosaic virus. This virus is one of the yellow mosaics [McKinney (4) and Jensen (1)] which was obtained by isolating from the central region of one of the bright yellow spots that sometimes develop on leaves infected with common tobacco mosaic. The virus obtained from this spot was diluted and rubbed on *Nicotiana glutinosa* leaves to produce local lesions and to enhance its purity. The virus from one of the lesions was then inoculated into Turkish tobacco and produced chlorotic spots at the infection points on the inoculated leaves. These later became necrotic. The systemic symptoms consisted of a distinct yellowing along the veins, appearing first in the youngest leaves and remaining in the mature leaves. These symptoms have remained constant during several transfers of the virus through Turkish tobacco plants. The symptoms produced by this mutant are similar or identical with those illustrated by Stanley (7) as due to a strain of tobacco mosaic called J14D1.

The infected leaves were collected 20 days after inoculation and were frozen at $-18°$ C. Purification was by the alternate high and low speed centrifugation method. The micrographs were taken with an R.C.A. Type B electron microscope. Both of these techniques have been described earlier (6).

Representative micrographs of common tobacco-mosaic virus and of the yellow mutant are in figure 1. The length distribution of the characteristic particles of the two viruses is shown in figure 2. It is evident that there were apparently no detectable changes in the size or appearance of the particle during mutation. Such changes as occurred during mutation were apparently not of a nature to be detected by means of the electron microscope.

Knight (2) has reported the rib-grass strain of tobacco-mosaic virus to have essentially the same particle size as tobacco-mosaic virus, and Knight

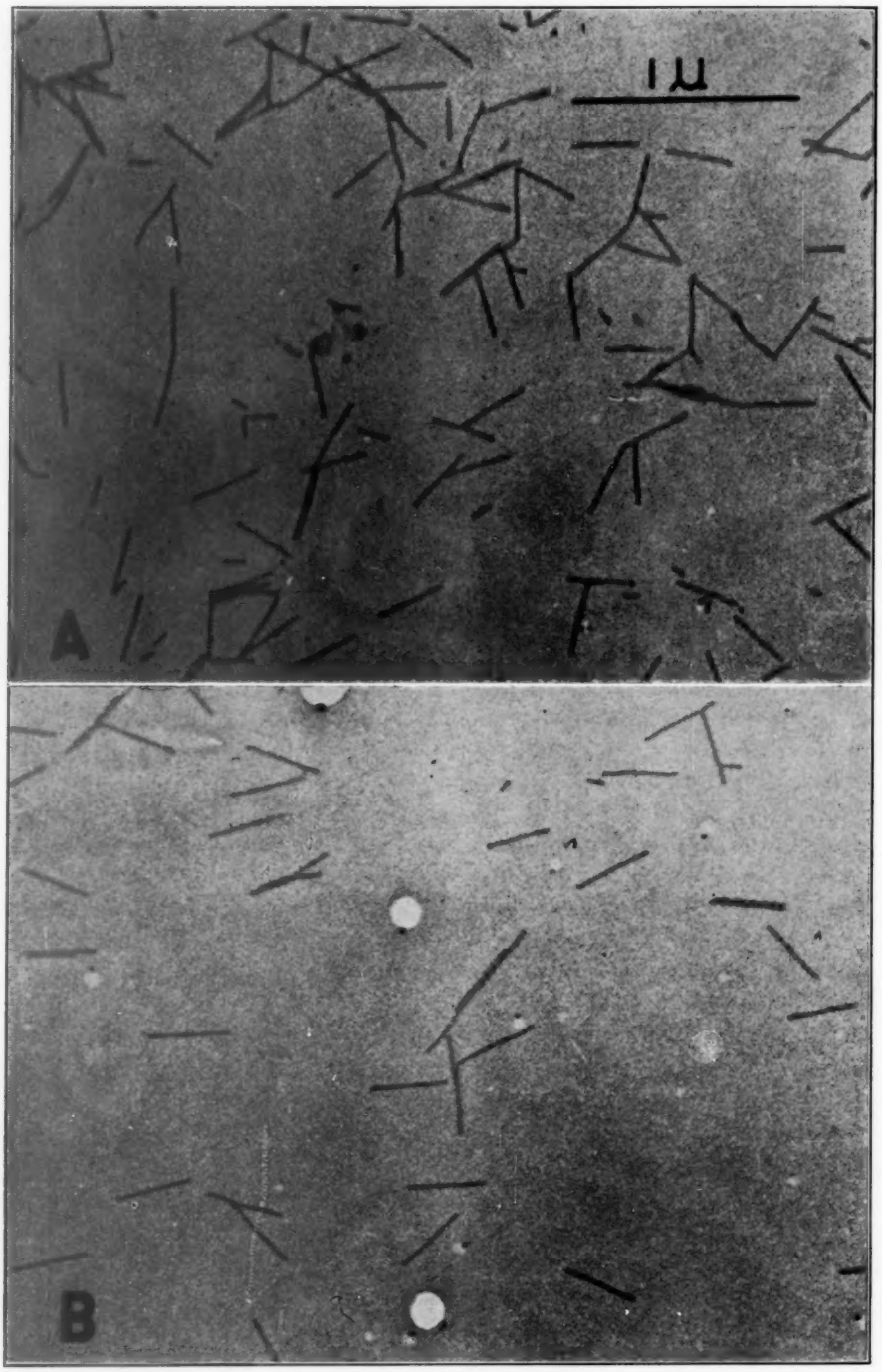

FIG. 1. Micrographs of two strains of tobacco-mosaic virus. A. Common tobacco-mosaic virus particles. B. Particles of a yellow mutant of tobacco-mosaic virus.

and Stanley (3) have reported cucumber virus 4 to have essentially the same size as tobacco-mosaic virus. They have also presented other evidence showing that cucumber virus 4 may be related to tobacco-mosaic virus. Unpub-

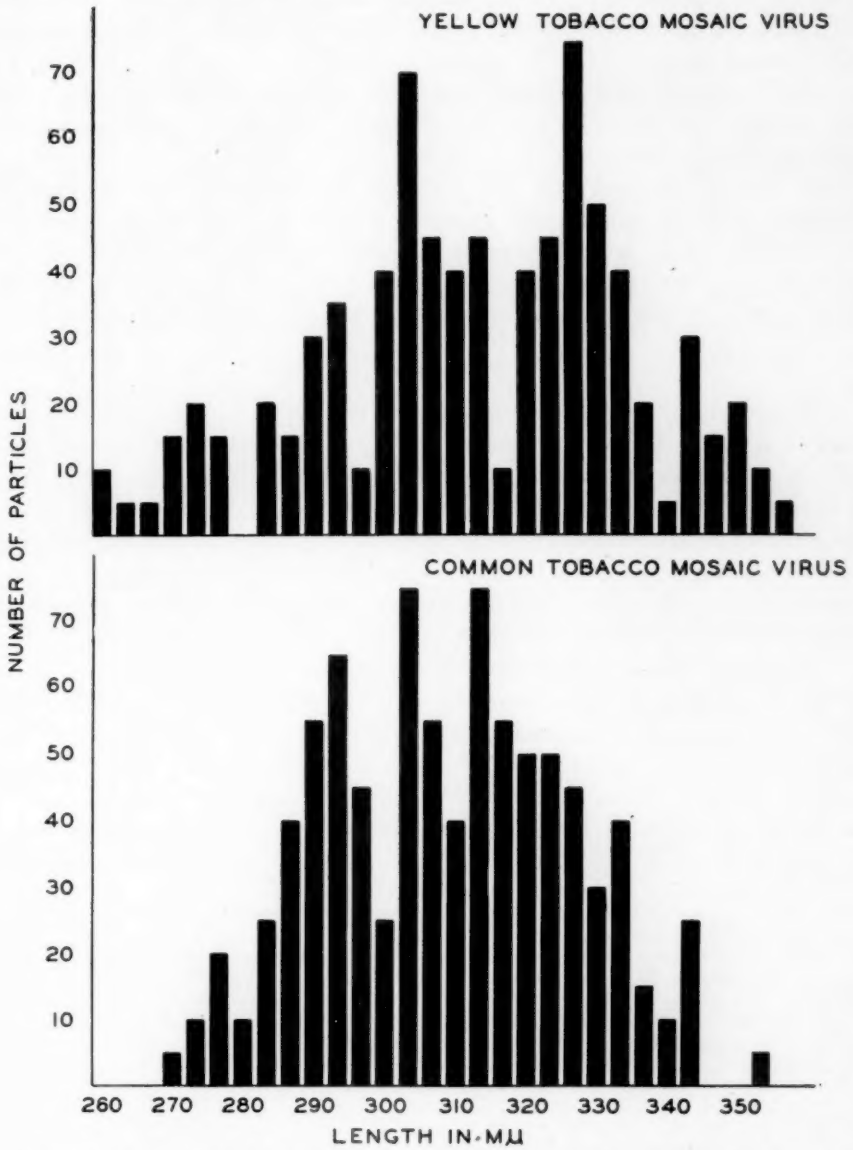

FIG. 2. Length distribution curves of common tobacco-mosaic virus and a yellow mutant of this virus.

lished results (9) indicate that the particles of 2 strains of potato X virus are usually between 500 and 600 mμ long and again are indistinguishable from each other in electron micrographs. However, the symptoms produced by these 2 strains on tobacco show little resemblance.

Our results and those of Knight, and Knight and Stanley indicate that a detectable fracture of the virus particle does not ordinarily accompany mutations of rod-shaped viruses. At present there is unfortunately little evidence to indicate whether the changes in amino acid content, that have been found to accompany certain virus mutations (7), are a cause or a result of the mutations.

The results reported and reviewed above indicate that the size of a properly purified rod-shaped virus is a valuable character in determining the relationship of viruses.

In view of the fact that the 2 strains of tobacco mosaic reported by Melchers *et al.* (5) are the only reported strains where mutation appears to be accompanied by a shortening of the virus particles, the question arises as to whether these viruses were really strains of tobacco mosaic or whether their short particles may have been due to the methods of preparation.

SUMMARY

Common tobacco-mosaic virus and a yellow mutant isolated from it were studied with the electron microscope. The yellow mutant was indistinguishable in size and form from the parent strain when isolated from plant juice. It is concluded from the combined available evidence that mutations of rod-shaped viruses are ordinarily not accompanied by a modification of the virus particle sufficiently great to be detected by the electron microscope. Accordingly, if an unidentified virus has the particle size of a known rod-shaped virus there is considerable likelihood that the 2 viruses are related.

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MILD RUSTY MOTTLE OF SWEET CHERRY (PRUNUS AVIUM)[1]

S. M. Zeller and J. A. Milbrath

(Accepted for publication September 30, 1946)

INTRODUCTION

Mild Rusty Mottle of sweet cherry trees has been observed in Oregon for the last six or seven years. It was at first believed to be the same as the severe type of Rusty Mottle described by Reeves (5) from Washington, but comparative studies have proved it to be distinct in several respects. Mild Rusty Mottle occurs in some sections of the states of Oregon, Washington, and Idaho. In Oregon it has been observed throughout the Willamette Valley, and in Curry, Hood River, and Wasco counties. Where surveys have been made one orchard at least 35 years old had 20 per cent Mild Rusty Mottle while another orchard about 25 years old had 32 per cent of the trees infected (9). In some orchards, it has been estimated that more than 50 per cent of the trees were infected. On the other hand, some of the oldest orchards have little or none of the disease, and some young orchards may be relatively free of Mild Rusty Mottle, while still others originating from different nursery stock have only scattered trees infected from the start (3).

Although trees affected with Mild Rusty Mottle continue to produce fruit for a number of years, the loss from this disease in northwestern sweet cherry orchards is undoubtedly of considerable economic importance.

HOST RANGE AND SUSCEPTIBLE VARIETIES

Mild Rusty Mottle seems to infect one variety of sweet cherry about as readily as another. It has been found in the orchards affecting trees of the Bing, Black Republican, Black Tartarian, Lambert, and Napoleon varieties. It was also isolated from Montmorency sour cherry. A number of other stone fruit species and varieties have been found by inoculation to be susceptible. These will be discussed under transmission studies.

SYMPTOMS

In sweet cherries. From a distance one may distinguish trees affected with Mild Rusty Mottle by their general yellowish greenness as compared to the dark lush greenness of healthy trees. The whole tree takes on a rusty or bronzed appearance by late June or early July. Older infected trees often show considerable die-back and unthriftiness, indicating a probable increase with age in susceptibility to unfavorable climatic factors, such as drouth and low temperatures. Affected trees, however, seem to decline more rapidly than healthy trees, as indicated by poorer terminal growth, set of fruiting spurs, etc., although their decline is much slower than that brought about by Severe Rusty Mottle. The disease may be found in a single branch, but by the next season it may have spread to the whole tree.

[1] Published as Technical Paper No. 488, with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany.

There are no particular leaf symptoms very early in the spring. Usually in May or early June the mottling first appears in leaves which have attained a certain maturity some distance back from the growing tips. There are no symptoms in the terminal growth throughout the season.

The mottling starts as yellowish or merely light green areas in the leaves. These areas take on various forms. If circular, the centers are lighter yellow than the margins (Fig. 1, A), the latter gradually becoming bronzed or reddish (Fig. 1, B). This bronzing or rustiness shows first on the upper leaf surface, then gradually shows reddish below. It borders the areas whatever their shape or size. The bronzing or rustiness at times, however, is in the form of tiny stipples. This stippling may be only on, or very close to, the

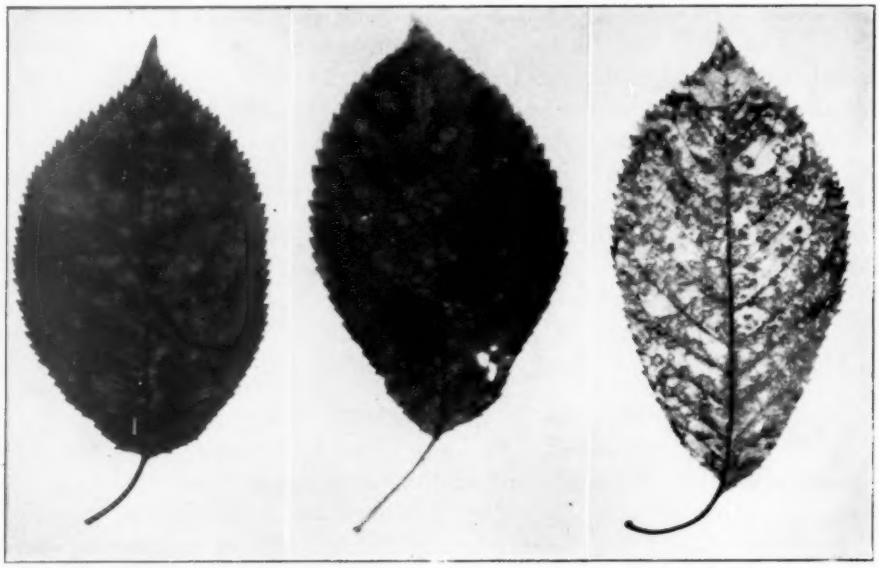

FIG. 1. Three leaves illustrating stages in the development of leaf symptoms of Mild Rusty Mottle in Bing cherry; collected June 12, 1942, The Dalles, Oregon. A. Circular chlorotic areas constitute the first leaf symptoms. B. Rusty or bronzed margins form around the chlorotic areas. Such symptoms persist until late fall. C. A small percentage of the oldest leaves may take on a bright yellow rusty-mottled appearance and drop during June.

veins before it shows in the mesophyll. In such cases it usually starts at the base of the leaf blade. If the rustiness follows a line pattern or rings, there is usually a feathered out appearance, at least on one side. At times the leaves that come out early or are shaded toward the center of the tree may become chlorotic with a severe bright yellow or whitish mottle (Fig. 1, C). Such leaves are shed rapidly, but such shedding does not result in a general defoliated appearance of the tree. In unusual cases certain infected trees may have a rather heavy loss of relatively greenish mottled leaves even before fruit-harvest time, but the next year the same trees may have no particular leaf cast during the summer. Leaf symptoms of Mild Rusty Mottle differ little, if any, in different varieties of sweet cherries.

Fruits of trees infected with Mild Rusty Mottle are somewhat retarded in ripening. In the Napoleon variety the fruits are inclined to be clear yellow and usually without the red cheeks of healthy fruit.

In sour cherries. Montmorency sour cherry trees from which the Mild-Rusty-Mottle virus was obtained, had considerable die-back and a peculiar leaf symptom, neither of which has been definitely proved to be expressions of this particular disease. The leaves had a mild mottle and they were much smaller than on healthy appearing trees. The under surfaces of the leaves were mottled with a rusty coloration which seems to be brought about by necrosis of the lower epidermis in certain areas, and which may be limited to one side of the leaf, to the central area, or along the veins. A close examination of the rustiness shows it to start with a reddish necrosis along the veinlets. This spreads along these veins until the whole spot appears reddish to the naked eye. Finally the whole under surface of the leaf has a reddish mottle. By this time the upper surface appears mottled and necrosis begins in the older chlorotic spots.

TRANSMISSION STUDIES

Mild Rusty Mottle is perhaps as easily transmitted as any woody plant virosis. In every case where sweet cherry has been inoculated with sweet-cherry inoculum, transmission has been successful with or without apparent organic union of the two tissues. In all of our transmission experiments reported below, inoculation has been by summer budding. Sometimes, however, the inoculum has consisted of a mere shield of infected bark and wood slipped under the bark of the tree to be inoculated. Each graft is bound with a nurseryman's rubber band used in budding. In each experiment two to five trees of a variety were inoculated. The trees have ranged in age from one to five years from the nursery. There has been no indication of difference of reaction of young and older cherry trees to the Mild-Rusty-Mottle virus. All trees became uniformly infected the season following inoculation. Mild-Rusty-Mottle inoculum used in these studies came from the following localities: Benton, Hood River, Lane, Multnomah, Polk, Wasco, and Washington counties in Oregon, and Benton and Yakima counties in Washington.

SYMPTOMS IN INOCULATED TREES

More than 200 trees of several varieties of stone fruits have been inoculated with Mild Rusty Mottle and the results of these inoculations are reported rather concisely in table 1. Attention should be called to some of the peculiarities of the disease, as learned from the transmission studies.

In sweet cherries. The symptoms of Mild Rusty Mottle as obtained by graft inoculations are about equally conspicuous in the seven varieties of sweet cherries thus infected, unless it should be noted that sometimes in the leaves of Black Republican there is a golden, marbled mottle without particular bronzing or rustiness. None of the many sweet cherry trees inoculated with this disease has had leaves with any pronounced necrosis, such

as produced by the Severe Rusty Mottle described by Reeves (5, 6) and by the type of rusty mottle described from Utah by Rhoads (7, 8). Toward the end of summer, however, there may be slight necrosis following bronzing or rustiness. If this occurs it is usually in very small spots when compared with the large necrotic spots in leaves affected by Severe Rusty Mottle.

TABLE 1.—*The reactions of primary hosts to inoculation with the virus of Mild Rusty Mottle and the reactions of secondary hosts following inoculation from the primary host*

Source of virus, host inoculated, and reaction[a]		Source of virus, host inoculated, and reaction[a]	
From sweet cherry		From sour cherry	
To cherry		To cherry	
Napoleon	+	Napoleon	+
Bing	+	Bing	+
Black Republican	+	Black Republican	+
Lambert	+	Lambert	+
Mahaleb	−	Mazzard	+
Mazzard	+	Montmorency	+
Montmorency	+	To Bing cherry	+
Kwanzan	−	Amanogawa	−
To Bing cherry	+	Kwanzan	−
To Shirofugen cherry	−	Naden	−
Mt. Fuji	−	Shirofugen	−
Shirofugen	−	To peach	
Golden	+	Elberta	−
Deacon	+	J. H. Hale	−
Schmidt	+	Early Muir	−
To Prunus virginiana	−	To Rio Oso Gem peach	−
To peach		To Bing cherry	+
Elberta	−	To Black Republican cherry	+
Early Muir	−	Rio Oso Gem	−
To Bing cherry	+	To Bing cherry	+
To Black Republican cherry	+	To Italian prune	−
To Rio Oso Gem peach	−	To Bing cherry	+
To Coates plum	−		
To Burbank plum	−		
To French prune	−		
J. H. Hale	−		
To Early Muir peach	−		
To J. H. Hale peach	−		
To Bing cherry	+		
Rio Oso Gem	−		
Rochester	−		
To Italian prune	−		
To Bing cherry	+		

[a] The plus sign (+) indicates symptoms of Rusty Mottle in leaves. The minus sign (−) indicates the absence of symptoms. Some hosts on which symptoms were lacking acted as carriers of the virus.

In flowering cherries. Among the flowering cherries (*Prunus serrulata*) inoculated with Mild Rusty Mottle, only the Kwanzan and Naden varieties have shown any mottle with rustiness in the leaves, while the Amanogawa, Mt. Fuji, and Shirofugen varieties have had no leafy symptoms. The Mild-Rusty-Mottle symptoms in leaves of Kwanzan and Naden are, however, quite sparsely scattered over the tree or limited to certain areas of the tree, such as in single branches. In some instances even Kwanzan and Naden trees showed no symptoms, although infected with the virus. Latent viruses in

sweet cherries, of course, have always complicated the study of any other sweet-cherry viruses in flowering cherry varieties (4).

In sour cherries. The Mild-Rusty-Mottle virus was found to occur in Montmorency cherry naturally, and it was transmitted to cherries, peaches, and prunes (Table 1). The leaf symptoms in Montmorency cherry artificially inoculated with Mild Rusty Mottle from either sour or sweet cherry were essentially the same as those observed in Montmorency trees naturally infected. The leaves in which vein necrosis and the resulting bronzing appear in early season, drop later, and no leaf symptoms may be seen during the remainder of the season. No die-back has occurred in any of these trees, which were inoculated three years ago.

In seedling cherries. In general, inoculated Mazzard seedlings show the same symptoms of Mild Rusty Mottle as do standard varieties of sweet cherry; however, there is some difference in individual Mazzard trees. Some have more of the reddish bronzed coloration in the leaves while others have definite ring spots with bronzed margins, and some show a finely stippled mottle.

Mahaleb seedlings have shown no leaf symptoms of the disease, but they may be symptomless carriers.

In wild hosts tested. *Prunus virginiana* (Eastern Chokecherry) and *Osmaronia cerasiformis* (Indian Plum) were not affected by inoculations, but no attempt was made to recover the virus from inoculated plants.

In peaches. The study of Mild Rusty Mottle on peach trees has been difficult, because all sources of inoculum have also included one or more ring-spot (2) or other sweet-cherry latent viruses (4) in addition to the Mild-Rusty-Mottle virus. When such buds with this mixed inoculum were grafted into peach trees, the latter became severely dwarfed and rosetted, with thickened twigs and short internodes. The leaves were smaller and darker green than normal and without mottling. Somewhat similar symptoms have been observed in peach trees inoculated with sweet or sour cherry having only latent or ring-spot viruses.

Nearly 100 peach trees of several varieties were inoculated from infected sour and sweet cherry, but in no case has there been any symptom which could be attributed to Mild Rusty Mottle alone. Mild-Rusty-Mottle virus was recovered from inoculated peach trees. These are reported in table 1.

In prunes. When the disease was inoculated into prune and plum trees of several varieties, no leaf symptoms resulted. Italian prune was proved to be a carrier of the virus by transfer back to Bing cherry, but the other varieties have not been so tested.

SYMPTOMLESS CARRIERS

As may be observed in table 1, J. H. Hale, Early Muir, Rio Oso Gem peach, and Italian prune have been proved, by return inoculation to sweet cherry, to be symptomless carries of Mild-Rusty-Mottle virus. The majority of stone fruit varieties which do not show symptoms may prove to be symptomless carriers.

COMPARISON OF MILD RUSTY MOTTLE AND SEVERE RUSTY MOTTLE

These two diseases are distinct in several respects. These differences are reflected particularly by the degree of severity on trees, and in different expressions of symptoms in leaves, fruit, bark, and growth of branches.

The decline of old sweet cherry trees infected with Mild Rusty Mottle is apparently not nearly so rapid as that brought about by Severe Rusty Mottle. The fruits of sweet cherry trees infected with Mild Rusty Mottle are normal in size and quality, whereas those of trees of the same varieties affected by Severe Rusty Mottle are insipid and reduced in size. The roughened bark, mentioned by Rhoads (7) has never been observed in trees infected with Mild Rusty Mottle; however, three Bing trees inoculated with the type of Severe Rusty Mottle from Washington developed a superficial splitting of the bark on one-year-old wood.

TABLE 2.—*The reactions of primary hosts to inoculation with the virus of Severe Rusty Mottle and the reactions of secondary hosts following inoculation from the primary host*

Source of virus, host inoculated, and reaction[a]		Source of virus, host inoculated, and reaction[a]	
From sweet cherry		From Early Muir peach	
To Early Muir peach	+	To Nonpariel almond	−
Montmorency cherry	+	To Tilton apricot	+
Bing cherry	+	To Kwanzan cherry	+
To Amanogawa cherry	+	To Shirofugen cherry	+
To Kwanzan cherry	+	To Early Muir peach	+
To Mt. Fuji cherry	+	To J. H. Hale peach	+
To Naden cherry	+	To Rio Oso Gem Peach	+
To Lovell seedling peach	+	To Rochester peach	+
To Prunus virginiana	+	To Burbank plum	−
		To Coates plum	−
		To French prune	−

[a] The plus sign (+) indicates symptoms of Rusty Mottle in leaves. The minus sign (−) indicates the absence of symptoms.

Leaf casting of Bing cherry trees affected with Severe Rusty Mottle is usually sufficient to cause the trees to have a stripped, bare, or defoliated appearance (6). Such severe leaf cast has never been observed in trees infected with Mild Rusty Mottle; although there may be some leaf cast in early summer, the trees do not appear noticeably defoliated.

Severe necrosis of the chlorotic areas in leaves of sweet cherry trees infected with Severe Rusty Mottle is usually apparent by late spring or early summer, while in trees affected by Mild Rusty Mottle a necrosis other than bronzing is seldom, if ever, seen as a leaf symptom.

After about the first of June the leaf symptoms of Severe Rusty Mottle show on the leaves almost to the tips of the branches, while in Mild Rusty Mottle there are no leaf symptoms for some distance back from the tips. The leaf symptoms of sweet cherries infected with either of these diseases seem not to be influenced by changes in temperature, but hold throughout the season.

In certain hosts, such as peach varieties, chokecherry (*Prunus virginiana*), and some varieties of flowering cherry (*P. serrulata*), inoculated with Mild Rusty Mottle, there are no particular leaf symptoms, whereas the same varieties and species inoculated with Severe Rusty Mottle show the rustiness and bronzing in the leaves similar to that described in sweet cherries. (Compare tables 1 and 2.) In peaches the leaf symptoms of Severe Rusty Mottle start as a chlorotic mottling in circular areas with diffuse edges. The leaves gradually turn yellow to rusty orange with greenish islands. The earliest symptoms are sometimes quite like asteroid spot (1) and then go through much the same series of symptom development as in sweet cherries, except that there is no necrosis. Since this Severe-Rusty-Mottle inoculum proved to be free of sweet-cherry latent viruses, the symptoms described are apparently those of Severe Rusty Mottle. Tilton apricot gave the same symptoms as peach when inoculated with Severe Rusty Mottle. Flowering cherries react in much the same way as peaches and apricots.

It would perhaps be well to point out specifically the difference between Mild Rusty Mottle as it occurs in Oregon and Washington and the form which Rhoads has described in Utah (7, 8). Rhoads' suggestion that "even the meager occurrence of the necrotic spots associated with this disease constitutes a very reliable means of detecting it and that a diagnosis made on this basis will err mainly in being too conservative in some orchards," does not apply to Mild Rusty Mottle in the least. The Utah strain, however, may be a form of Severe Rusty Mottle.

NOMENCLATURE

Since there are so many differences between Mild and Severe Rusty Mottle there is considerable question as to the relation between the two. There seems to be but one characteristic in common, namely, the rustiness in the leaf symptoms in sweet cherry. This at least should be considered generic. But too little is known of the two forms to decide which is typical or which might be a variety of the other. Surely in most cases a plant is given specific rank if it differs in three or more characters from its nearest congener. In this case there are six or seven character differences, but at present we merely propose Mild Rusty Mottle as a common name and Severe Rusty Mottle as a common name of the form Reeves has described.

CONTROL

As is true with other diseases of this nature, the control in future orchards rests in the use of clean nursery stock. Where healthy trees are to be top-worked to other varieties great care should be exercised in the selection of budwood free of harmful viruses. In the established orchard where few trees are infected, the diseased trees should be removed at once. In cases where the removal of infected trees will too greatly deplete the established orchard, they may be allowed to remain until unprofitable. Where a thinning program is necessary, mapping of the diseased trees should precede

pulling, and the pulling of trees be so arranged as to remove the greater number of diseased trees.

SUMMARY

Mild Rusty Mottle is described as distinct from the severe form described by Reeves in 1940. It occurs in eastern and western Oregon, and in parts of Idaho and Washington. Trees affected with it persist for a number of years, but apparently are more subject to winter injury and drouth than healthy trees. The leaves show a rusty and bronzed mottle, but without the necrosis so prevalent in the severe form.

The disease has been found natively in sweet and sour cherry trees, and by graft inoculations has been transferred to sweet and sour cherry, to peach, to flowering cherry, and to Italian prune. The symptoms in various hosts are described. Symptomless carriers of the disease are all of the varieties of peaches tested and Italian prune. Infected Montmorency cherry is symptomless for most of the growing season. The differences between the mild and severe forms of rusty mottle are pointed out. Since these two viroses are so distinct, the common names, Mild Rusty Mottle and Severe Rusty Mottle, are proposed.

Control is through the use of clean scion wood in the nurseries and roguing out affected trees in orchards.

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FIELD METHODS OF TESTING FOR ROOT-KNOT INFESTATION[1]

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The testing of crop varieties for farm use, the breeding of crops for root-knot resistance, the evaluation of the root-knot infestations in soils and the comparison of different crops for resistance require a precise method of measuring the degree of infestation by the root-knot nematode, *Heterodera marioni* (Cornu) Goodey. Systems used in the past have generally lacked a simple method of expressing the results numerically and were, therefore, not subject to statistical evaluation. Accordingly, investigations were directed toward establishing a satisfactory means of expressing the degree of root-knot infestation obtained in field experiments.

Godfrey (5) reviewed the earlier literature on attempts to arrive at soil populations of the root-knot nematode, first by actual counting and identifying of nematodes in a soil sample, and later by root-gall counts on indicator crops. His studies with indicator crops showed a high correlation between the percentage of infested plants and the average number of galls per plant when such counts were low, averaging 1 to 30 per plant. The gall count and percentage count data were transformed by him to estimates of actual soil populations. At the present time the gall-count system is used extensively in detailed studies; but for field use, involving large numbers of plants, it is too slow and expensive. The rapid grouping system of McKinney (9), based on grouping of plants in classes depending on magnitude of disease, has become more widely used for handling field experiments. Guba (6) reported percentages of indicator plants with zero, light, moderate, and severe infection. Barrons (2) used 5 classes in studying root-knot resistance in bean and pea seedlings. His categories were: class 1, not infested; and classes 2 to 5 with increasing amounts of galls. At the beginning of the study standards representing each class were selected for comparing the remainder of the roots. Smith (11), employing 5 classes, graded cotton roots on a basis of percentages of the total root system showing galls. Newhall and Stark (10), Kincaid and Reeves (8), and Stark *et al.* (12) report work with root knot using several modifications of the grouping system.

The determination of resistance to infestation involves a consideration of the factors affecting the production of disease symptoms. Where the root-knot nematode is concerned, plants have all degrees of resistance ranging from immunity to very high susceptibility. Apparently root-knot-resistant plants are not resistant because of any ability to prevent the entrance of

[1] Cooperative investigations between the Division of Cotton and Other Fiber Crops and Diseases and the Division of Nematology, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Dept. of Agriculture, the Georgia Agri. Exp. Sta. and the Coastal Plain Exp. Sta. Paper No. 147, Journal Series, Georgia Agricultural Experiment Station.

larvae from the soil (3), but rather through the lack of ability of the larvae to grow to maturity within the roots. In a highly resistant plant, such as *Crotalaria spectabilis*, larvae enter and even form small and inconspicuous knots, but never develop to maturity (1). The same condition was noted by Christie and Albin (4) for several other species of plants tested against various strains of root-knot nematodes. With certain exceptions reported by Steiner (13), notably freesia and cyclamen, root-knot nematodes form more or less conspicuous knots on the roots of host plants. Since the degree of infestation of a given plant which has been in the soil for several months will be somewhat related to the number of progeny produced by the original invaders of the plant, variability in egg production is a factor of resistance. If few or many eggs are produced, the end result will be a plant lightly or heavily infested, respectively. It seemed apparent that resistance in typical cases could be evaluated by observing the gall formation on plants which had been grown to maturity (or near maturity) in soil heavily infested with root-knot nematodes.

DIFFERENTIATION OF VARIETIES

The differentiation of crop varieties in regard to root-knot resistance was studied, using soybeans and cotton. Four experiments with soybeans and four with cotton were conducted over a 3-year period, 1941 to 1943. The tests were at Tifton, Plains, and Perry, Georgia. At Tifton, one experiment with soybeans was on soil artificially contaminated with root-knot nematodes by burying infested roots. The remainder of the tests were on naturally contaminated soils. Seed from the same lot of soybeans were used in all tests. The cotton seed were from different lots in different years. Replications consisted of single-row, randomized plots long enough to contain approximately 100 plants. Soybeans were planted the first week of May and cotton the first week in April. These are approximately the normal planting dates for both crops. Soil temperatures in April are somewhat low for normal nematode activity but are more favorable in May at the time of planting soybeans.

The plants were allowed to grow to maturity (September and October) in the various experiments to permit the recording of yield data, then dug and examined. In digging, an effort was made to remove a large proportion of the root system, but no effort was made to get all of it. The roots of all plants were examined by visual inspection for the presence of root-knot galls, and each plant was classified according to degree of infestation. The categories were similar to those used by Smith (11); Class 0 = all roots without visible galls, Class 1 = 1 to 25 per cent of roots with galls, Class 2 = 26 to 50 per cent, Class 3 = 51 to 75 per cent and Class 4 = 76 to 100 per cent of roots with galls. This system was used for recording data obtained from all tests at Plains and Perry. Similar infestation categories were used for data obtained from the Tifton tests except that arbitrary standards were set up for each class as reported by Barrons (2). Noninfested plants were

placed in Class 0, those with light infestation in Class 1, those with very heavy infestation in Class 4, and those with intermediate degrees of infestation in Classes 2 and 3.

The two systems are summarized in table 1.

Data were calculated in all instances by the McKinney (9) formula as modified by Horsfall and Heuberger (7) who applied it to a defoliation disease of tomatoes. It is as follows:

$$\text{Disease index} = \frac{\Sigma \text{ category numbers}}{\text{No. plants} \times 4} \times 100$$

The summarization of category numbers is obtained by multiplying the number of plants in each category by their respective category numbers from 0 to 4, and adding the products.

It is proposed that "root-knot index" apply to infestation data obtained by the first of the two methods, using the above formula. Thus the "root-knot index" becomes an estimate of percentage infestation with a range from zero to 100 and comparable to other disease data based on percentage of infection. To indicate the index obtained by the second method as used in the Tifton experiments it is suggested that "relative index" or "relative root-knot index" be applied. By this latter system the indexes are relative only to others in that specific study since new standards are adopted for each experiment.

TABLE 1.—*Categories established for the determination of root knot by the root-knot index system and by the relative index system*

Infestation category	Root-knot index system[a]	Relative index system[b]
0	No infestation	No infestation
1	1 to 25 per cent	Light
2	26 to 50 per cent	Medium
3	51 to 75 per cent	Heavy
4	76 to 100 per cent	Heavy

[a] Used in tests at Plains and Perry.
[b] Used in tests at Tifton.

RESULTS OF SOYBEAN VARIETY TESTS

Root-knot indexes, relative root-knot indexes, and least significant mean differences for the soybean variety tests are given in table 2. Examination of the data indicates three distinct resistance groups are represented by the five varieties. Laredo was more resistant in all cases, Biloxi and Otootan were intermediate, and Clemson and Georgian were most susceptible. Only the 1942 Tifton test gave clear-cut separation of all three groups. In the 1941 Tifton experiment, on artificially infested soil, the Clemson variety was in the group with Biloxi and Otootan. The two 1941 field tests likewise have one variety each which is not clearly differentiated. The 1942 test at Tifton, with a lower requirement for significant differences, segregates the varieties more fully than the remainder of the studies. This may be attrib-

uted largely to the use of 6 replications as compared with 4 or 5 replications in the other tests, and a more uniform distribution of nematodes in the soil. Field infestations of root-knot nematode are generally spotted and even with small tests the results suggest a minimum of 6 replications may be necessary for most locations.

The method of recording data did not affect the conclusions that might be drawn from individual tests. The root-knot index system used in the

TABLE 2.—*Root-knot indexes and relative indexes of soybean varieties, 1941–1942*

Variety	Series						Varietal mean
	1	2	3	4	5	6	
Artificially infested soil—Tifton, 1941[a]							
Laredo	21	19	25	28	.....	.....	23.2
Biloxi	59	43	36	55	.....	.....	48.2
Otootan	30	39	78	57	.....	.....	51.0
Clemson	66	44	49	79	.....	.....	59.5
Georgian	68	84	86	94	.....	.....	83.0
L.S.M.D. (0.05)	.....	.....	.....	.....	.....	.....	20.0
Naturally infested soil—Tifton, 1941[a]							
Laredo	3	5	25	8	9	.....	10.0
Biloxi	32	20	18	15	48	.....	26.6
Otootan	27	23	14	27	40	.....	26.2
Clemson	36	36	19	51	70	.....	42.4
Georgian	49	66	54	26	56	.....	50.2
L.S.M.D. (0.05)	.....	.....	.....	.....	.....	.....	16.6
Naturally infested soil—Plains, 1941[b]							
Laredo	8	26	21	5	2	.....	12.4
Biloxi	27	51	59	34	42	.....	42.6
Otootan	46	89	62	39	29	.....	53.0
Clemson	96	100	99	76	18	.....	77.8
Georgian	94	52	92	80	63	.....	76.2
L.S.M.D. (0.05)	.....	.....	.....	.....	.....	.....	24.5
Naturally infested soil—Tifton, 1942[a]							
Laredo	9	12	6	13	6	9	9.1
Biloxi	30	20	17	19	22	40	24.7
Otootan	27	25	34	20	37	51	32.3
Clemson	30	58	50	39	54	41	45.3
Georgian	57	46	61	37	37	65	50.5
L.S.M.D. (0.05)	.....	.....	.....	.....	.....	.....	11.0

[a] Relative root-knot indexes. [b] Root-knot indexes.

Plains test resulted in a wider spread between the most susceptible and most resistant variety than was found by the relative index system used in the two Tifton tests on naturally infested soils. A wider spread would be of value in differentiating a large number of entries. However, the relative index spread obtained by using a set of standards is affected by the standards selected. With added experience in the adoption of standards the full index range might be utilized.

RESULTS OF COTTON VARIETY TESTS

The cotton variety tests were conducted in a manner similar to the soybean tests. Six replications were used, except at Perry where data were recorded from only three. The "index" was obtained at the Plains and Perry locations and "relative index" at Tifton. The results (Table 3) indicate Coker 4 in 1-5 to be the only variety with outstanding resistance. Rhyne's Cook was significantly more resistant than Stoneville 2B in the 1942 Plains and Tifton tests and also more resistant than Coker 100 Wilt-1 in the Tifton test. Early Wilt has lower index numbers than several varieties in the 1941 and 1942 tests but was significantly more resistant than other varieties in only one study, the 1942 Plains test. Differences among varieties in the 1943 Perry test were found nonsignificant and the data are shown only to emphasize the importance of utilizing sufficient replications in the presence of such large inter-plot variability.

TABLE 3.—*Root-knot indexes of cotton varieties*

Variety	Plains, 1941	Plains, 1942	Tifton,[a] 1942	Perry, 1943
	Index	*Index*	*Index*	*Index*
Coker 4 in 1-5	62.0*[b]	59.7*	24.3*	47.7
Rhyne Cook	87.5	86.3*	40.8*	57.0
Early Wilt	91.3	83.0*	50.0	36.0
Station 21	97.8	94.8	52.0	63.3
Stoneville 2B	93.5	98.2	56.0	73.7
Coker 100 Wilt-1	94.7	93.5	56.5	61.3
L.S.M.D. (0.05)	12.3	10.8	13.9	.....[c]

[a] Tifton data are relative indexes.
[b] Starred (*) figures indicate that a variety was significantly more resistant than one or more other varieties.
[c] Differences not significant.

This group of varieties may be considered as representing the range in root-knot resistance in upland cotton varieties commonly planted in the Southeast (11). All, except Coker 4 in 1-5, are relatively susceptible and they tend to group at the upper range of susceptibility as illustrated in the Plains 1941 test in which 4 of 6 varieties have indexes varying from 91.3 to 97.8. The same 4 varieties have relative indexes varying from 50.0 to 56.5 in the Tifton 1942 test. In these studies with cotton varieties there appears to be no preference between the two methods used for recording data. However, minor differences in gall size were noted in the 4 most susceptible varieties and in order to utilize these differences the relative index may be preferable. Relative index utilizes both size and number of galls in evaluating susceptibility, whereas, the root-knot index is based on numbers and largely disregards size of galls.

ROOT-KNOT RESISTANCE OF DIFFERENT CROPS

A number of crops grown in rotation with cotton were planted at Plains, April 10, in a soil where root knot of cotton had previously occurred. The

TABLE 4.—*Root-knot indexes of several different interplanted crops. Plains, Georgia, 1945*

Crop	June 29	July 21	October 20
	Index	*Index*	*Index*
Cotton (Deltapine)	37.9	55.7	100
Corn (Whatley's)	77.1	75.8	Mature, roots decayed
Sorghum (Texas Seeded Ribbon)	9.5	30.2	Older roots decayed
Soybean (Mamloxi)	35.5	34.1	No apparent increase
Soybean (Biloxi)	21.9	16.0	do
Peas (Brown Crowder)	100.0	86.2	Killed by root knot, Aug. 1 to 20
Lespedeza (Korean)	71.5	95.2	do Aug. 15 to 30
Lespedeza (Kobe)	44.8	68.0	do Sept. 15 to 30
Vetch (Hairy)	100.0	100.0	All plants dead July 1
Velvet bean	7.5	0.0	0.0

test consisted of single-row plots, 50 feet long, with 4 replications. Parts of each plot were dug on June 29 and July 21 with the remainder being left for final observation on October 20. The development of the infestation (Table 4) could be followed by the root-knot indexes measured on different dates. Brown Crowder peas, Kobe and Korean lespedeza were very susceptible and died before maturity and seed production. Hairy vetch was the most susceptible crop tested, although it was not apparent whether the killing was due to root knot or partially due to hot weather. With soybeans there was no tendency for rapid increase of root knot as the season progressed but with the rather tolerant cotton, infestation continued to increase to the end of the season. Velvet beans were lightly infested early. In later diggings, due to the vigorous, deep-growing root system, few small roots were found and galls were not observed. Fibrous-rooted crops such as corn and sorghum are quite tolerant to root knot. The greater resistance of sorghum is illustrated by the lower index. Older roots of sorghum were decaying from fungal attacks and were continuously replaced by newer ones, and final data on root knot were not obtained.

CONTROL STUDIES WITH NEMATOCIDES

Use of the root-knot index system of recording infestation data in soil-fumigation studies is illustrated in table 5. This study was conducted in

TABLE 5.—*The effect of different rates of application of D-D and chloropicrin on root-knot infestation in tomatoes. Plains, Georgia, 1944*

Nematocide	Pounds per acre	Spacing of injection points	Amount injected	Root-knot index
		Inches	*Ml.*	
D-D	192	36 × 18	7.5	41.1
D-D	256	36 × 18	10.0	21.8
D-D	385	18 × 18	7.5	10.3
D-D	512	18 × 18	10.0	0.0
Chloropicrin	535	18 × 12	5.0	22.2
Check	.....	.....	.....	84.7
L.S.M.D. (0.05)	.....	.....	.....	25.0

cooperation with the "Experiment Station Committee" for investigation of new nematocides. The soil treatments were applied April 25 and data were recorded October 26, 1944. Marglobe tomato plants were used as the test crop. The root-knot indexes illustrate the control and lasting effects of different rates of application of D-D[2] in comparison with chloropicrin. Since the maximum number of plants per plot was 6 in this test the requirement for significance is relatively higher than might have been obtained with larger populations.

DISCUSSION

A comparison of the "root-knot index" and "relative index" systems for determining varietal resistance gave essentially similar results from location to location, from year to year, and by different workers. Under conditions of the studies conducted neither system was preferable to the other but there was some observational evidence that where larger numbers of crop varieties are being tested under conditions of severe infestation the relative index may be preferable. The index system otherwise permits a closer comparison between different studies since it is based on percentage infection, whereas, arbitrary standards are utilized for the relative index.

Data collected at Plains and Tifton were generally in good agreement, indicating that there is little difference between the host preferences of the root-knot nematodes at those two locations. It should be emphasized that, in the light of the data presented by Christie and Albin (4), this might not be true for other locations. Of 13 nematode populations tested by them, only one gave a heavy infestation on cotton, one gave a light infestation, two a very light infestation, one no infestation, and the other eight gave only a trace of infestation with no eggs produced. On Laredo soybeans 10 populations were tested by Christie and Albin (4) and 2 of the nematode populations failed to infest at all, 2 more had only a trace of infestation with no eggs, 3 had a very light infestation, and 3 a light infestation which might correspond to that found at Plains and Tifton in the study reported here. Until more is learned concerning the number of races of root-knot nematode and their locations, data on resistance of hosts of the root-knot nematode should be considered as applying only to the locations specified, and not as being of general significance. On the other hand, continued testing of resistance, if such a plan as the one here presented is followed, will provide the data for conclusions as to the habits and locations of the various races of root-knot nematodes. It would also provide information of great practical value for the planning of rotations.

The lack of uniform method of recording infestation data in relation to studies on soil fumigation is illustrated in cooperative reports and other recent publications (8, 10, and 12). Studies on a regional basis, involving a wide range of infestation intensity with the accompanying need for com-

[2] D-D is a mixture of dichloropropylene and dichloropropane containing a small percentage of more highly chlorinated compounds.

parison and integrating with related experiments, probably may best be evaluated by use of the root-knot index rather than the relative index.

Experiments reported here were not designed to answer the question concerning number of replications or number of plants necessary to obtain the results desired; however, some information was obtained. With crop varieties the number of replications used varied from 3 to 6. The evidence indicates that, due primarily to the extreme variations in root-knot larvae populations in the soil, 6 replications are preferable to a smaller number. Three replications were not sufficient to obtain significant differences between any of the varieties at the Perry location. In the studies reported here the number of plants per plot used for determining varietal differences was arbitrarily placed at 100. This number is perhaps greater than necessary, 40 or 50 in most instances would give a satisfactory plot reading. For the studies with nematocides 4 to 6 plants per treatment gave significant differences between rates of applications of D-D. However, larger numbers of plants are undoubtedly necessary for more exact information. Godfrey (5) concluded that from 25 to 100 indicator plants per block were sufficient to give a satisfactory estimate of the root-knot infestation, the accuracy of estimates being greater, of course, with the larger numbers.

SUMMARY

A system for testing root-knot resistance of plant varieties in the field has been developed. At least 6 replicated plots for each variety are located on soil known from previous examination to have a heavy infestation of root-knot nematodes. Plantings are made when soil temperatures are favorable for activity of the nematodes and the plants are allowed to grow to maturity. Roots are then removed, examined, and grouped, using the McKinney system. Disease indexes are then calculated, using the Horsfall and Heuberger modification of the McKinney system.

Two systems of determining root-knot infestation grades were compared for differentiating soybeans and cotton: one, based on the percentage of the root system with visible galls; the second, based on the adoption of standards for each root-knot class with the remainder of the roots graded by comparison with the standards selected. It is proposed that "root-knot index" be used to designate infestation indexes obtained by the percentage method and "relative root-knot index" used for designating infestation indexes obtained by comparison with adopted standards. The two systems were about equally effective in differentiating varieties. The consistency of the results from year to year and location to location indicates the validity of both methods.

Root-knot index data were obtained on several interplanted crops for evaluating their possibilities in a root-knot-reducing rotation. The seasonal development of infestation was demonstrated and the crops segregated for root-knot reaction.

Possibilities for use of the root-knot-index were demonstrated in relation to studies on chemical control.

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FACTORS AFFECTING INFECTIVITY, SPREAD, AND PERSISTENCE OF PIRICULARIA ORYZAE CAV.[1]

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INTRODUCTION

Piricularia oryzae Cav., the cause of the "blast" disease of rice, is found in nearly all the rice-growing regions of the world. In the United States it is found principally in the rice-growing areas of Arkansas, where it annually causes some damage to the Japanese-type rice varieties.

The effect of various environmental factors upon the infection of rice by *Piricularia oryzae* has been considered by several investigators. Hemmi (8) and Suzuki (17, 18) found that plants grown in dry soils were more susceptible to infection than plants grown in wet soils. Tochinai and Komuja (21) found that a 3-day exposure to drought prior to inoculation was sufficient to increase the amount of infection and that an exposure for an equal length of time to a saturated atmosphere resulted in reduced infection. It was shown by Abe (2) that rice seedlings were most susceptible to infection when grown at low soil temperatures (20° C.) and least susceptible when grown at higher soil temperatures (28–32° C.). Several authors (14, 18, 20) found that susceptibility increased as the amount of nitrogenous fertilizer was increased. Abe (1) demonstrated that infection was more intense when seedlings were kept shaded or in darkness. Imura (12) studied the development of lesions on seedlings kept under various degrees of light intensity. He found that the initial development of the lesions occurred most rapidly on those plants which were kept slightly shaded but that the maximum enlargement finally occurred on those given the most light. Sakamoto (13) found a marked increase in infection after the plants were exposed to strong winds. This was attributed to wounds on the leaves due to rubbing or possible physiological changes in the leaf tissue due to drying. Tochinai and Komuja (21) also found that plants exposed to injury were more susceptible. According to Abe (3), no infection takes place at relative humidities below 90 per cent because of the failure of spores to germinate. Hemmi and Imura (11) found that air humidities in excess of 89 per cent were necessary for conidium formation. Hemmi and Abe (9) found that air temperatures from 24° to 28° C. favored the rapid development of the initial stages of leaf infection and, at these temperatures, a minimal period of from 6 to 8 hours of continuous wetting of the plants was necessary to establish infection.

[1] Work conducted at Camp Detrick, Frederick, Maryland, and at Beaumont, Texas, from June, 1944, to August, 1945.

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Investigations reported in this paper deal with varietal resistance, effect of age of plant on infection, environmental factors affecting primary and secondary infection, longevity of conidia, reduction of disease development by *Helminthosporium oryzae* van Breda de Haan, and susceptibility of other cereals. The field experiments were at Beaumont, Texas.

MATERIALS AND METHODS

Source and Production of Inoculum

Two field isolates of *Piricularia oryzae* were obtained from Arkansas. The one was isolated from dead rice stubble in 1943 and the other from Cody rice in 1944. Both of these isolates, and re-isolates obtained by their passage through the host, were used in the present study.

The conidia used for inoculation of the host were produced on a grain substrate containing equal parts by weight of Hegari sorghum and oats. A satisfactory method for preparing the substrate was to add 100 g. of each grain and 230 ml. of water to a 2800-ml. Fernbach flask. The grain was presoaked for 2 hours and then autoclaved for 20 min. at 15 lb. pressure on each of 3 successive days to insure complete sterilization. The substrate was seeded with a 10-ml. spore suspension obtained from stock cultures on 2 per cent rice-polish agar and containing at least 200,000 spores per ml. The seeded substrate was incubated at room temperature for 6 to 7 days and was aerated at a rate of 5 ml./min./g. of dry substrate for the entire period. The spores were then removed from the substrate by washing the whole in distilled water. The resulting suspension was used for inoculation of the host, or finely ground, black, neutral peat was incorporated with the suspension, the mixture filtered, the filter cake dried at 40° C. for 24 hours, powdered, and the resulting dust used as inoculum.

Inoculation of Plants

All the inoculations were made with the aid of hand-made dusters and atomizers (Fig. 1, B and C). The dusters were capable of dispensing from 1 to 10 g. of dust inoculum per min. with air pressure supplied by a hand atomizer bulb or a compressor. The atomizers had a dispensing capacity of 50 to 75 ml. of suspension per min. with air pressure applied by the compressor. Lower rates of application could be achieved by reducing the air pressure.

Considerable difficulty was encountered in securing uniform distribution of leaf infections on rice plants when the latter were inoculated with aqueous suspensions of conidia. The effectiveness of these suspensions was improved by the addition of 0.05 per cent sodium oleate and 0.25 per cent gelatin, making it possible to obtain a 4- to 10-fold increase in the number of leaf infections per plant and a more even distribution of leaf infection (5). Hence, in all cases where conidial suspensions were used for plant inoculations, the conidia were suspended in a sodium oleate-gelatin solution.

It was found that fresh conidia applied at a rate of 1 million in 10 ml. of suspension per 6-inch pot of 15 plants (3–5-leaf stage), provided sufficient inoculum for maximum infection. On an area basis, maximum infection occurred following the application of a conidial suspension applied at a rate of 1.0 million conidia per sq. ft.; and an application of 2.5 million conidia per sq. ft. was necessary if the inoculum was applied in the dry form using the duster. These rates were used throughout the experiments.

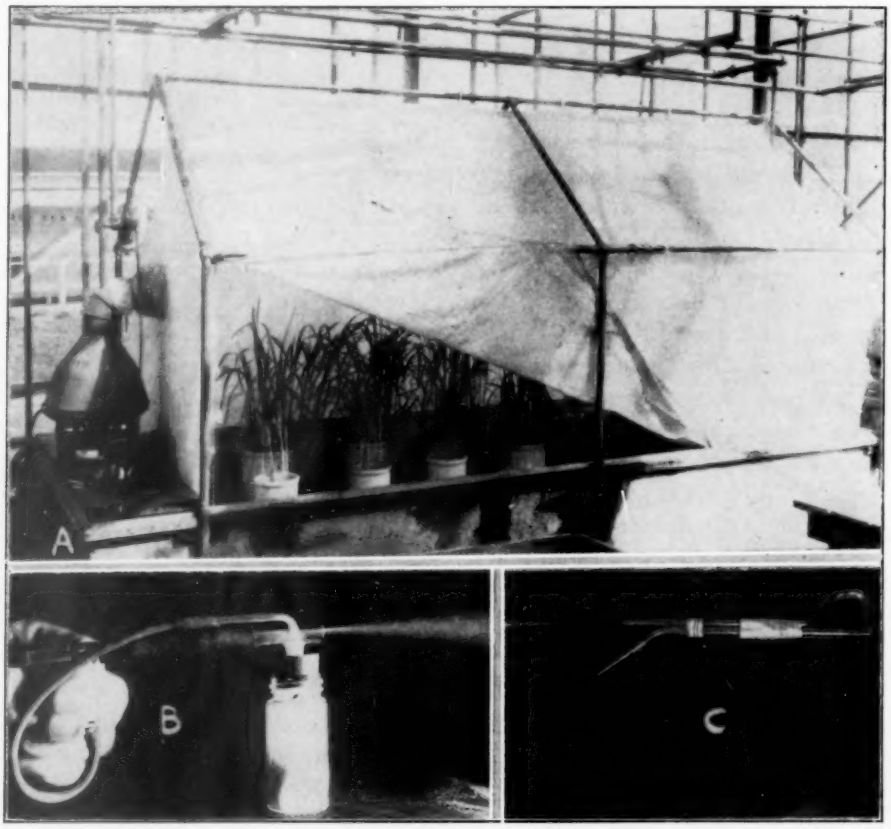

FIG. 1. A. Humidity tent containing rice plants in gallon and half-gallon glazed pots. Note overhead water spray in operation and Standard Humidifier Mod. 31 at end of tent. B. Duster used to apply dry conidial inoculum. C. Sprayer used to apply conidial suspension. The upper tube is attached to the compressor; the lower tube is inserted in the suspension.

Establishment of Infection

A humidity tent was used for providing favorable conditions for the establishment of *Piricularia oryzae* infection on host plants (Fig. 1, A). This tent consisted of a muslin cover supported by a steel frame over a deep, water-tight bench. An external overhead sprinkling system was used for keeping the muslin wet as an aid in maintaining humid conditions inside the tent. A water humidifier, set at one end of the bench, maintained a

high humidity within the tent so that the plants were covered with free moisture for as long a period as desired. Required temperatures (22–28° C.) were maintained in the tent by controlling the room temperature and the temperature of the water in the bottom of the bench. A constant record was maintained of the air temperature in the tent by means of a recording thermograph. Sufficient air circulation was produced by the humidifier to enable a study of secondary spread of *P. oryzae* under controlled conditions.

Evaluation of Results

The basic criteria, unless otherwise stated, for the evaluation of *Piricularia oryzae* infection on rice in the seedling (3–5-leaf) stage of development were (1) the number of leaf spots produced, (2) the number of leaves infected, and (3) the number of leaves killed. The death of individual plants was not used as a criterion since it rarely resulted from primary infection. It was only when the plants were subjected to repeated inoculations or placed under ideal environmental conditions for reinfection that they were actually killed by *P. oryzae*. The leaf lesion and infected leaf counts were made as soon as the spots could be distinguished and before they had begun to coalesce. This was generally from 4 to 9 days after inoculation. A count of the dead leaves was made approximately 2 weeks after inoculation.

HOST FACTORS RELATED TO INFECTION

Varietal Susceptibility

To insure rapid and conclusive results in greenhouse tests, several varieties of rice were required which were very susceptible to *Piricularia oryzae*. Seed of 17 Japanese short-grain and 2 American medium-grain varieties were secured and all were tested in the seedling (3–5-leaf) stage. Their relative susceptibility was estimated from (1) the rapidity of lesion development, (2) the size of the lesions, and (3) the killing of the leaves. On this basis the varieties were placed in 5 groups, as follows:

1. *Very susceptible:* Characterized by very rapid enlargement of lesions and killing of many of the leaves, with size of lesions not restricted. Onsen, Acadia, Wataribune, Caloro, Asahi, Wasa Shinriki, Colusa × Blue Rose, and Blue Rose.

2. *Susceptible:* Lesions enlarged more slowly than in group 1, resulting in the killing of some of the leaves, with size of lesions not restricted. Kinai 195, Hattan, and Guneki.

3. *Moderately susceptible:* Lesions large but definitely restricted, with only a small number of leaves killed. Haya Ozeki, Kameji, and Gin Bozu (C.I. 6355).

4. *Moderately resistant:* Lesion size restricted to 1–2 mm., no leaves killed. Akaho, Early Wataribune, Gin Bozu (C.I. 6873), and Meshibu.

5. *Resistant:* Infection readily established but with lesions remaining as small flecks on the leaves. Butte.

On the basis of this classification, and because of availability, Acadia and Onsen varieties were selected for greenhouse studies. The latter variety was favored because it was possible to secure seed free from *Helminthosporium oryzae*.

Stage of Plant Development

The age of the plant was an unreliable criterion of the stage of development of rice plants because of variation in the growth at various times of the year. Consequently, tests were carried out according to the stage of development of the plant as follows: (1) seedling, (2) tillering, (3) booting, (4) heading.

In order to have plants in various stages of development available at the same time, a series of Acadia and Onsen rice plantings was begun in the greenhouse in July, 1944, and new plantings were made at approximately 2-week intervals thereafter until the oldest plantings had reached the flower-

TABLE 1.—*Susceptibility of various parts of the rice plant at various stages of development*[a]

Tissues infected	Stage of plant development and age in weeks at time of inoculation			
	Seedling	Tillering	Late tillering and booting	Heading
	1–4	4–7	7–11	11–13
Leaves and leaf sheaths	X	X	Trace	0
Leaf and sheath junction	X	X	X	X
Nodes of panicle	.....	.....	X	X
Culm	.....	.....	0	X
Panicle[b]	.....	.....	X	X

[a] X = infection; 0 = no infection.
[b] Panicle infections include those of its branches, spikelets, lemma, and palea.

ing stage. All the plants were sprayed with an aqueous suspension of *Piricularia oryzae* conidia to determine their relative susceptibility to infection at the various stages (Table 1). It is apparent from these results that only plants in the seedling and tillering stages were susceptible to leaf and sheath infection, with the degree of resistance to these infections increasing as the plant became older. From the late tillering to the late booting stage the plants were very resistant to infection. It was not until they reached the late booting and heading stages that they again became susceptible to infection. During these later stages all the exposed parts of the panicle were subject to infection which resulted in "blasting." Completely blasted panicles resulted from infections of the nodes, culms, or entire heads, whereas partially blasted panicles resulted from infections of some of their individual parts.

Abe (4) studied the comparative susceptibility of the different portions of the rice plant to *Piricularia oryzae* and found that the greatest number

TABLE 2.—*Percentage of blasted panicles and grains resulting from inoculations with Piricularia oryzae at various stages of development*

Variety[a] and stage of development at time of inoculation	Noninoculated plants (control)				Inoculated plants			
	Panicles	Grains	Filled grains	Light grains	Panicles	Completely blasted panicles	Grains	Blasted grains[b]
			Pct.	*Pct.*				*Pct.*
Acadia								
Heading (flowering)	9	704	96	4	8	3	641	82
Early heading	10	740	89	11	10	9	810	93
Late booting-early heading	7	563	95	5	8	5	533	65
Booting	8	391	95	5	5	3	233	71
Onsen								
Flowering; soft dough	9	702	93	7	8	7	672	94
Booting; milk	6	627	91	9	7	7	499	100
Late tillering; flowering	9	631	90	10	9	5	536	82
Tillering; late booting and early heading	6	400	86	14	5	3	336	93

[a] Acadia variety was inoculated once. Onsen variety received 2 inoculations at 2-week intervals.
[b] Includes those grains which ordinarily would be light. (Note percentage of light grains in control.)

of leaf lesions, on plants in the 3–5-leaf stage, occurred on leaves "of medium size," and that the lesions on the youngest or oldest leaves were relatively few. He also noted that the number of diseased spots per unit length of leaf was greatest at the middle part. On the other hand, the number of lesions per unit length was greater near the tip than at the basal part of the young and medium leaves. On the older leaves the opposite relationship was observed. He concluded that "the most important factors which control distribution of the diseased spots on leaves seem to be a tendency of leaves to get wet (Benetzbarkheit), the angle of leaves to the halm axis and the degree of development of the mechanical tissue of leaves." Somewhat similar observations were made by the authors on plants which had been inoculated with an aqueous conidial suspension. However, a more uniform distribution of leaf lesions was obtained by suspending the conidia in a sodium oleate–gelatin solution at the time of inoculation.

Abe (4) also noted, in the case of grown plants, that the disease outbreak occurred at the greatest frequency on the spikelets, but that this type of infection resulted in only a negligible reduction in yield; whereas infection "on all parts of the pedicels of spikes and the second internode of halms tended to show the greatest influence on the yield of rice kernels. . . ." In general, these observations agree with those made by the authors except that under controlled conditions severe infection of the spikelets resulted in a high reduction in yield, especially when the heads were inoculated during the early flowering stage.

Suzuki (19) concluded from his studies on the anatomical differences between susceptible and resistant plants that "the thickness of the outer walls and of the silicated outermost layer as well as the number of the silicated bulliform cells, of the silicated long or short cells, and of the silicated stomata seems to be closely correlated with the susceptibility of the rice plant to blast disease, while the number of stomata is not considered to be in close correlation with the susceptibility of the plant to the disease." It was not determined whether the increased resistance noted in the leaves and sheaths of plants in the later stages of development, as observed in our work, was of the same nature as that observed by Suzuki (19).

The plants which were inoculated in the later stages of development were grown to maturity and the panicles harvested to determine the percentage of blasting that had occurred (Table 2). The Acadia variety received only one inoculation while the Onsen variety was inoculated a second time approximately two weeks later to simulate secondary spread. It is evident from these results that *Piricularia oryzae* is capable of causing extensive damage to a rice crop which is in its later stages of development.

ENVIRONMENTAL FACTORS RELATED TO INFECTION

Period of Continued Wetness of the Plants

This environmental factor is a result of an interaction of several other factors such as relative humidity, air temperature, dew point, etc. It has

been considered to be one of the most important factors governing the establishment of primary infection and secondary spread in a rice field. Hemmi and Abe (9) found in their experiments that continuous exposure to wetness for 6 to 8 hours at temperatures between 20° and 28° C. was necessary for the infection of rice seedlings.

In order to determine the period of continued wetness necessary for the establishment of initial infection on rice at temperatures optimum for the growth of the fungus (24–28° C.), a series of potted seedling rice plants in the 3–5-leaf stage was inoculated with a suspension of conidia of *Piricularia oryzae* and placed in the humidity tent. Duplicate pots were removed at periodic intervals from 4 to 24 hours after inoculation. The number of lesions per infected leaf was determined after 5 days and the number of dead and infected leaves after 14 days (Table 3). These results were

TABLE 3.—*The influence of length of exposure to continued wetness on the amount of infection by Piricularia oryzae*[a]

Time in tent	Dead leaves per plant	Infected leaves per plant	Leaf spots per infected leaf
Hours	*Number*	*Number*	*Number*
4	0.0	0.0	0.0
8	0.0	0.0	0.0
10	0.0	0.5	1–3
12	0.0	1.2	1–10
14	0.1	1.3	1–10
16	0.1	2.1	1–10
18	0.3	1.7	1–10
20	0.2	1.5	1–10
24	0.2	0.9	1–10
(control)	0.0	0.0	0.0

[a] Data represent averages from 30 plants in each treatment.

similar to those found in previous experiments at slightly lower temperatures (20–22° C.), when 10 hours was the shortest time of exposure that resulted in infection. At these lower temperatures a period of 20 to 24 hours was necessary for the establishment of maximum infection. It was concluded that 16 to 24 hours of high humidity and continued wetness of the plants at 24–28° C. would cause maximum infection.

When it was discovered that a 16- to 24-hour period was necessary for the establishment of maximum infection on plants if the spores were applied directly, it was presumed that a longer period would be necessary for natural secondary spread to take place. The period of continued wetness necessary for secondary spread and the establishment of infection on rice at 24–28° C. was determined by exposing noninfected rice plants together with infected plants in the humidity tent. In one of these experiments, 4 pots of noninfected Onsen rice were placed in the center of the humidity tent and several infected plants were placed at each end with 2–3 feet between the healthy and infected plants. The 4 pots of healthy plants and the infected plants were exposed for 24 hours to continued wetness and to the air currents pro-

duced by the humidifier. At the end of the 24 hours the 4 pots were removed and replaced by 4 other pots of healthy rice. The water was turned off and the plants were exposed only to the air currents from the humidifier. After a 24-hour exposure all the original infected plants were removed and the 4 remaining pots of rice were exposed to 24 hours of continued wetness. The first series, which was exposed to continued wetness and secondary spread for 24 hours, had an average of 2.8 infected leaves per plant with leaf lesions too numerous to count. The second series, which was exposed under dry conditions of secondary spread, had an average of 0.3 infected leaves per plant, and there were very few lesions on these leaves. Thus it seemed that the conidia spread from the diseased to the healthy plants more readily during the wet period than during the dry period.

TABLE 4.—*The influence of time of exposure to continued wetness on the spread of Piricularia oryzae from infected to noninfected rice plants*

Time exposed	Additional exposure to high humidity	Number of plants	Leaf spots per plant	Infected leaves per plant
Hours	*Hours*		*Av.*	*Av.*
3	0	36	0	0.0
5	0	34	0	0.0
7	0	29	0	0.0
9	0	34	0	0.0
11	0	32	0	0.0
13	0	38	Trace	Trace
15	0	34	1–10	0.3
21½	0	32	50+	2.6
3	18	33	Trace	Trace
5	18	36	Trace	Trace
7	18	36	1–10	0.1
9	18	38	0	0.0
11	18	37	1–10	0.2
13	18	34	1–10	0.3
15	18	33	10–20	0.5
21½	18	32	50+	2.2

The same infected plants that served as a source of inoculum in the previous experiment were placed at both ends of the humidity tent and 32 pots of healthy Onsen rice seedlings were placed in the center of the tent. Sets of 4 pots were removed from the center of the tent after exposure for the time intervals given in column 1 of table 4. The plants that served as a source of inoculum were discarded at the end of 21½ hours. Twenty-four hours after the last pots were removed, 2 from each set of 4 were replaced and subjected to 18 additional hours of continued wetness (Table 4). Very little secondary spread occurred during the first 15 hours of exposure to continued wetness even when the exposed plants were resubjected to conditions favorable for infection. Since the most infection was established in 21½ hours, it would indicate that an exposure to continued wetness for an equal or greater length of time would be necessary in the field for maximum secondary spread and probable establishment or initiation of an epiphytotic.

The results obtained in the greenhouse were confirmed by an experiment in Texas on initial infection and subsequent secondary spread. *Piricularia oryzae* was not present naturally in the proximity of the tests. A 12×12 foot plot was selected in a field of Onsen rice and was inoculated with a conidial suspension of *P. oryzae* using the same rate of application as in the greenhouse experiments. The plants were in the seedling–early tillering stage. Immediately after inoculation the plants were exposed to a 14-hour period of continued wetness by dew and water of guttation which was sufficient to establish heavy infection. Lesions resulting from secondary spread were evident on rice plants at distances of 100 to 200 feet from the original inoculated plot within 3 weeks. The infection resulting from secondary spread was limited to very few leaf spots, and even in the plot itself infection was more or less comparable to the amount of spread that took place in a 10–15-hour period in the controlled humidity tent in the greenhouse. Proof of the spread was established by taking samples of infected leaves and placing them in a moist chamber to sporulate. After the rice ripened, 4 one sq. yd. areas were harvested from the plot and 4 similar areas were selected about 25 feet from the plot and harvested for controls. An average of 179 grams of rice was recovered from each sample area in the treated plot as compared to 260 grams from the control areas. Although there was a 100 per cent variation between replicate samples, it appeared that the pathogen was at least partially responsible for the reduction in yield. Thus it appeared that one reason for the absence of *P. oryzae* in the Beaumont, Texas, rice area was the lack of conditions favorable to secondary spread.

Periodic Wetting

No infection by *Piricularia oryzae* occurred when the inoculated plants were kept in the humidity tent for 8 hours or less (Table 3). Periods of continued wetness in nature are often less than 8 hours. It then seemed desirable to learn the effect on the amount of infection a relatively short period of wetting would have when it was followed later by a period of wetting sufficient in itself to allow the establishment of infection. Even though no infection occurred with 8 hours' exposure to continued wetness, it was known that the conidia would germinate on the leaf of the host within this time.

In order to determine the effect of short periods of wetting upon the establishment of infection, 14 pots of rice containing 15 plants per pot were each inoculated with a suspension of conidia of *Piricularia oryzae*. Duplicate pots were used for each treatment. The plants were inoculated with the same spore suspension at different times so that after exposure to continued wetness for desired lengths of time, they were all removed from the tent and placed on a greenhouse bench in the sun at 8 A.M. Ten hours later all the plants were replaced in the humidity tent for an additional 18 hours (Table 5). Plants exposed to wetting for 8 hours or less (followed by a dry and a second wet period) had 30 to 50 per cent of the amount of

infection obtained with the 10-hr. period. The greater amount of infection with the 10 and 12 hours of initial wetting was probably due to (1) infection already established when the drying began, and (2) the killing of some germinated conidia by drying on those plants exposed for shorter periods.

Period of Reinfection

From the standpoint of secondary spread in the field, it was of interest to determine the length of time elapsing between the establishment of initial or primary infection and the development of lesions on which sporulation would occur, thus providing a source of secondary inoculum. In addition, an attempt was made to determine the period of greatest conidial production and subsequent secondary spread from the new lesions. This was accomplished by inoculating a series of 40 pots of rice plants in the seedling

TABLE 5.—*The effect of periodic wetting of the plants on infection by Piricularia oryzae*

Initial period of wetting[a]	Av. no. dead leaves per plant	Av. no. infected leaves per plant	No. lesions per infected leaf
Hours			
2	0.1	0.9	1–5
4	0.0	0.7	1–5
6	0.1	0.9	1–5
8	0.0	0.7	1–5
10	0.7	1.8	1–10
12	0.4	1.3	1–10

[a] All plants had 10 hours of dry atmosphere, then 18 hours of wetting after the initial period of wetting. See table 3 for results obtained when plants received only the initial period of wetting.

stage and placing them in the humidity tent for 24 hours to establish primary infection. Duplicate pots from this series were replaced in the humidity tent at regular intervals after the initial inoculation to determine to what extent the infected plants would serve as sources of inoculum for their reinfections. The lesions on all those plants replaced between 6 and 10 days after the initial inoculation produced abundant conidia which resulted in the establishment of much secondary infection. No secondary infection occurred on plants replaced after 2 and 4 days. After 10 days, little secondary infection occurred and the plants were able to outgrow the initial infection. Thus it appeared that a minimum period of about 6 days after infection was necessary for the lesions to develop to the stage at which they were capable of producing conidia which in turn acted as inoculum for secondary spread.

Longevity of Conidia

One of the factors which determines the amount of infection that can be expected is the ability of the conidia to survive on plants in the field. The importance of this factor was determined by inoculating a series of 76 pots of Onsen rice and placing them on the greenhouse bench. One half of the

series was inoculated with a conidial suspension and the other half with a dry conidial dust. Four pots of rice from each series were placed in the humidity tent for a 24-hour period at daily intervals for the first four days and at 2-day intervals for the next 8 days. *Piricularia oryzae* developed only on those plants placed in the humidity tent from 1 to 6 days after inoculation. Thus conidia were able to remain viable on dry plants for 6 days under greenhouse conditions.

Sueda (15) suggested that conidia would survive fairly long when floating on the surface of the water in a rice field, but that when submerged they would die within two weeks. This introduced the question of the importance of irrigation water on the spread of *Piricularia oryzae* in the field. It appeared difficult actually to study the survival of the conidia in the field, so an experiment was conducted in the laboratory to determine the extent of survival of conidia in suspension at various temperatures (Table 6). In

TABLE 6.—*The viability of Piricularia oryzae conidia after storage at four temperatures for varying lengths of time in water suspension*

Time in days	Temperature in degrees C.			
	8	20	28–30	32
	Percentage germination[a]			
0	.....	.....	99	.....
0.25	100	99	100	99
1	97	91	38	9
2	98	52	5	0
3	100	22	1	1
5	100	5	1	1
8	100	0	0	0
14	80	0	0	0

[a] Based upon 100–250 conidia for each sample.

this experiment 50-ml. samples of conidial suspension containing 90,000 conidia per ml. were taken from a 7-day-old oats-sorghum culture and placed in 22-mm. test tubes. One tube was stored at each of the following temperatures: 8° C., 20° C., room (28–30° C.) and 32° C. At periodic intervals the tubes were shaken and samples taken for germination tests.

The viability of the conidia decreased rapidly during the first 24 hours in water suspension at 28–32° C. At 20° C. the rapid decline in viability was between 24 and 48 hours. After 8 days' storage at 8° C., the viability was 100 per cent but dropped to 80 per cent after 14 days in storage. Irrigation water temperatures in Texas were recorded for one week, in the early morning, at noon, and in the evening. The lowest temperature recorded was 31° C. and the highest 38° C. During this same period the daily minimum air temperature was 22° C. and the maximum 35° C. Thus it appears that few conidia would be able to survive in irrigation water more than 24 hours.

While the work with *Piricularia oryzae* was in progress, it was constantly observed that conidia did not germinate in suspensions unless they were

floating on the surface or were suspended in a shallow layer of water on a glass slide. This would indicate the probable lack of sufficient oxygen for germination. It was also observed that the conidia floating on the surface of water nearly always germinated if the temperature of the water was above 20° C. Taking these results and observations into consideration, it seems highly improbable that the irrigation water would serve as a common method for the dissemination and spread of conidia of *P. oryzae*.

Inhibition by Helminthosporium oryzae

Aoki (7) reported the suppression of germination of *Piricularia oryzae* conidia in contact with conidia of *Helminthosporium oryzae*. Hemmi *et al.* (10) stated that the presence of *H. oryzae* conidia resulted in a reduction of both germination and length of germ tube of *P. oryzae* when the two pathogens were applied simultaneously to rice plants. The present investi-

TABLE 7.—*The apparently inhibitory effect of Helminthosporium oryzae on Piricularia oryzae infection*

Inoculum	Replicate	*P. oryzae* lesions[a]	
		Total	Average
P. oryzae conidia	1	337	
	2	332	370
	3	442	
P. oryzae conidia plus *H. oryzae* mycelium	1	7	
	2	59	42
	3	49	
P. oryzae conidia plus *H. oryzae* conidia	1	5	
	2	15	14
	3	21	

[a] Each number represents lesions on 15 plants.

gation showed no evident antagonism between the two organisms when grown together on rice-polish agar or when the conidia of each were allowed to germinate together in water. In several experiments using the two pathogens together on plants, no definite conclusions could be drawn because of the erratic host response to inoculations with *P. oryzae*. In one experiment, however, it was shown that *H. oryzae* did reduce the development of *P. oryzae*. In this experiment a series of Onsen plants was inoculated in the greenhouse with ground up dried mycelium from *H. oryzae*, dried *H. oryzae* conidia, *P. oryzae* conidia, and with mixtures of the latter with the two forms of *H. oryzae* inoculum. In all cases *H. oryzae* infection was heavy. The number of *P. oryzae* lesions on plants inoculated with the two agents was markedly reduced below that where *P. oryzae* was used alone (Table 7). Although experiments showed no evident antagonism between the two pathogens when grown together on rice-polish agar or when the conidia of both were allowed to germinate together in water, the development of *P. oryzae* was reduced by the presence of *H. oryzae* when plants were inoculated simultaneously with the two fungi.

Other Cereal Hosts

Anstead (6) presented experimental evidence of the ability of *Piricularia oryzae* from rice to attack wheat. Sundararaman (16) reported successful cross inoculation of *P. oryzae* from rice to oats, ragi (*Eleusine coracana*), and wheat, and vice versa. In the following experiments, wheat, oats, barley, corn, rye, and sorghum seedlings were inoculated with a conidial suspension of *P. oryzae,* and placed in the humidity tent for 24 hours. Lesions appeared on barley, corn, rye, and wheat from 5 to 7 days after inoculation. Sorghum and oats had no lesions. Infected leaves of barley, corn, rye, and wheat were placed in glass moist chambers and examined 24 hours later. Conidia were produced in abundance on all the lesions examined on all four species. It was not ascertained whether these cereals were susceptible to *P. oryzae* in the field.

DISCUSSION

Because of the importance of the "blast" of rice in Asiatic countries, many of the factors influencing infection have been investigated and reported upon in the literature. Several significant points were demonstrated by the investigations reported in this paper.

The question of relative susceptibility of plant parts at different growth stages indicates that the inoculations should be made during the seedling stage because even susceptible varieties become resistant in the late tillering and early heading stages. Information in regard to susceptibility to panicle infection would have to be obtained from inoculations in the heading stage or as a result of secondary infection initiated from lesions established during the early stages of development.

Infectivity was also considered from the standpoint of the pathogen. In this case the moisture relationships were of major importance, especially the effect of continued wetness of the plants. It was found that 16 to 24 hours of continued wetness were necessary for the establishment of maximum primary infection at temperatures between 22° and 28° C., and in no case was infection established on plants in less than 10 hours. If the plants were subjected to periodic wetness, infectivity was held to 30–50 per cent of the maximum when the initial exposure period to continued wetness was 8 hours or less.

The period of continued wetness of the plants also was an important factor with respect to secondary spread. Experimental evidence obtained in greenhouse studies indicated that only a few conidia were liberated while the plants were dry. On the other hand, when they were kept wet the amount of secondary spread was extensive after 24 hours of exposure. Thus it appears that plants had to be wet before conidia would be liberated to infect other healthy leaves and plants. In addition, it was shown that only a slight amount of secondary spread would take place within a 15-hour period from infected plants which already had conidia on primary lesions, and that approximately 21½ hours of continued wetness were necessary for heavy infection.

These results may possibly explain the difficulties encountered while attempting to study the infectivity and spread of *Piricularia oryzae* at Beaumont, Texas, during the summers of 1944–45. Primary infection was readily established in the field by artificial inoculation of Onsen rice in its early stages of development. This was because of the nightly occurrence of heavy dews, which generally lasted from 12 to 14 hours, and favorable seasonal temperatures. It was expected that a correspondingly heavy amount of secondary spread would take place after heavy primary infection was established. This was not the case. The actual number of leaf lesions was few. Even when the plants reached the heading stage there was only a slight amount of culm and head infection. Knowing the factors necessary for optimum secondary spread, it appeared that the one favorable environmental condition lacking in Texas was the presence of continuously wet weather for periods greater than 24 hours. The daily weather conditions in Texas were such that as much as 24 hours of continued wetness was a rare occurrence in 1945 (with the possible exception of hurricanes). Thus, plants were never exposed to more than 12–14 hours of free moisture at a time. There was considerable rain, but only in showers followed by sunshine so that the plants dried immediately afterward. Apparently the conditions in the Beaumont area are not favorable for the secondary spread of *P. oryzae*.

One other consideration of importance was the presence of *Helminthosporium oryzae* infection in the rice in Texas. In greenhouse experiments, where the two pathogens were used together to inoculate plants, *H. oryzae* reduced the development of *Piricularia oryzae*. During the early part of the season of 1945, *H. oryzae* infection was light and was not considered of importance in reducing the spread and infectivity of *P. oryzae*. It may have been of considerable importance during the latter part of the season when the plants were in the flowering stage. At this time, the rice was thoroughly infested with *H. oryzae* and difficulty was encountered in establishing infection by *P. oryzae* even in the heading stage.

There appears to be no reason to believe that *Piricularia oryzae* conidia will survive for any great length of time in the irrigation water of the field. In laboratory studies, conidia stored in water at 32° C. lost their viability in 1 day; at 28–30°, in 2 days; and at 20° C., in 3–4 days. Those conidia that float on the surface of the water germinate within a few hours. Therefore, it seems highly improbable that the spores would survive for any length of time in irrigation water. In rice-growing areas there is nearly always heavy dew formation every night. This would mean that spores would be exposed to periodic wetness, and that too would tend to reduce the viability of the conidia.

SUMMARY

1. Rice plants were most susceptible to *Piricularia oryzae* in the seedling, early tillering, and heading stages.

2. Exposure of inoculated plants to continued wetness for 16 to 24 hours

at temperatures between 24° and 28° C. resulted in maximum *Piricularia oryzae* infection. No infection resulted from exposure of less than 10 hours.

3. Inoculated plants exposed to a wetting period of 8 hours or less followed by a dry and a second wet period, had from 30 to 50 per cent of the maximum amount of infection.

4. High humidity and the presence of free moisture on the leaves of infected plants were essential for secondary spread and the establishment of infection of rice plants. Under such conditions a 15-hour exposure was not sufficient to allow secondary infection to any significant degree, but a 21½-hour period resulted in secondary spread and the establishment of a high degree of infection.

5. A minimum period of 6 days after inoculation was necessary for lesions to develop to a stage capable of producing conidia which, in turn, acted as inoculum for secondary spread.

6. Conidia remained viable on dry plants for 6 days in the greenhouse. In suspension, the viability decreased rapidly the first 24 hours under storage at temperatures between 28° and 32° C. Since these were below the normal temperatures of irrigation water in Texas rice fields, it is believed that conidia would not be able to survive in irrigation water for more than 24 hours and thus it seems improbable that the irrigation water would serve as a common method for the dissemination of conidia of *Piricularia oryzae*.

7. No antagonism was noted between conidia of *Piricularia oryzae* and *Helminthosporium oryzae* when they were grown together on rice-polish agar or when the conidia of each were allowed to germinate together in water. When the two were combined and used for plant inoculations, *H. oryzae* reduced the amount of disease produced by *P. oryzae*.

8. Eighteen short-grain and 2 medium-grain rice varieties were tested in the seedling (3–5-leaf) stage for their relative susceptibility to *Piricularia oryzae*. Of these, 11 were classified as being very susceptible or susceptible, one as resistant, and the other intermediate in reaction.

9. Barley, corn, rye, and wheat in the seedling stage of development were found to be susceptible to infection by *Piricularia oryzae*.

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SOYBEAN DISEASES IN ONTARIO AND EFFECTIVENESS OF SEED TREATMENT[1]

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Co-incident with a rapidly increasing acreage of soybeans in southwestern Ontario during recent years has been the discovery of a corresponding increase in the number and diversity of the diseases that affect this crop. To what extent seed treatment might prove efficacious in controlling certain of the seed-borne diseases of the district seemed to the writers to be one of the problems warranting immediate investigation. Consequently, studies in this connection and along related lines were undertaken in 1943, and the results obtained during the past three seasons are reported in the present paper.

Soybeans were first grown in Canada about 50 years ago (6, 17). For many years they attracted comparatively little attention as is shown by the fact that until 1940 the average acreage in the Dominion was only 10,000 acres (6). In 1942, however, the acreage increased more than fourfold to 41,490 acres yielding a total of 912,000 bushels (19). In 1944, the soybean crop in Canada totalled approximately 45,000 acres, of which 44,700 yielding 845,000 bushels valued at $1,690,000 were grown in the province of Ontario (6, 20). Thus, to date, commercial production of soybeans has been confined almost entirely to Ontario, where conditions are most favorable for their growth and where prospects for marketing them on a basis of large-scale production have greatly improved (2). In Ontario, production of soybeans is not uniformly province-wide, for of the 1944 total of 44,700 acres, 35,400 acres were concentrated in the counties of Essex, Kent, Middlesex, Norfolk, and Lambton in southwestern Ontario.

In 1926, Drayton (7) reported the occurrence of three diseases of the soybean in Canada. The number of parasitic diseases now known to have been found in soybean-growing areas variously scattered across the Dominion from Nova Scotia to British Columbia, has reached a total of 13,[3] which figure represents approximately half of the known number of parasitic diseases of the soybean. Because of the fact that large-scale production of soybeans is more concentrated in southwestern Ontario than elsewhere in Canada, it is of especial interest and significance that (a) all 13 diseases of the soybean known to occur in Canada have been found within this relatively restricted area (Table 1), (b) 8 of them have not yet been reported

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[3] Eleven of these diseases have been variously reported in the Annual Reports of the Canadian Plant Disease Survey. An additional two, as yet unreported, namely, stem rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, and purple speck, or lavender spot, caused presumably by *Cercosporina kikuchii* Mats. and Tomo. have been found by the writers, and specimens are on deposit in the laboratory herbarium.

as occurring elsewhere in the Dominion, (c) 10 of them have been found within the area since 1942 (4), and (d) at least 8—possibly 9—of them are known to be seed-borne (Table 1).

TABLE 1.—*Parasitic diseases of soybeans reported as occurring in southwestern Ontario*

Common name	Causal organism	Date first reported	Seed-borne	Not yet reported elsewhere in Canada
1. Bacterial leaf spot or bacterial blight	*Pseudomonas glycines* (Coerper) Stapp	1924	+	
2. Soybean mosaic	Soja virus 1	1924	+	
3. Downy mildew	*Peronospora manchurica* (Naoumoff) Sydow	1935	+	
4. Anthracnose	*Colletotrichum glycines* (Hori)	1942	+	
5. Phyllosticta leaf spot	*Phyllosticta sojaecola* Massl.	1943	+	
6. Pod and stem blight	*Diaporthe phaseolorum* (Cke. and Ell.) Sacc. var. *sojae* (Lehm.) Wehmeyer	1942	+	+
7. Fusarium blight	*Fusarium oxysporum* Schl. f. *tracheiphilum* Snyder and Hansen	1942		+
8. Frog-eye	*Cercospora sojina* Hara	1943	+	+
9. Brown spot	*Septoria glycines* Hemmi	1943	+	+
10. Bud blight, top necrosis, streak	Virus of tobacco-ring-spot group	1944		+
11. Charcoal rot	*Macrophomina phaseoli* (Maubl.) Ashby	1944		+
12. Stem rot	*Sclerotinia sclerotiorum* (Lib.) de Bary	1942		+
13. Lavender spot or purple spot	(Presumably) *Cercosporina kikuchii* Mats. and Tomo.	1942	?	+

LITERATURE REVIEW

Pioneering in North America in seed treatment of soybeans, Wolf and Lehman (28) in 1926 and Lehman (16) in 1929 reported that certain of a large number of fungicides under test increased the stand of plants and gave good control of downy mildew, whereas others reduced the stand and failed to control such diseases as frog-eye leaf spot and bacterial pustule.

In greenhouse trials in Oklahoma in 1941 and 1942, Davy (5) found that treatment of Virginia soybean seed with New Improved Ceresan and Spergon effectively prevented seed rots and pre-emergence damping-off in soil naturally infested with *Rhizoctonia solani* Kühn.

In a field test in Minnesota in 1942, Tervet (25) obtained no clear evidence that seed treatment with New Improved Semesan Jr. and Spergon controlled any of the seed-borne diseases.

In 1943, Koehler (13) called attention to coordinated interstate greenhouse tests, preliminary results of which indicated that when germinability of soybean seed is low as a result of too high a moisture content, emergence can be increased by treatment with Ceresan, Spergon, or Arasan.

According to Johnson and Koehler (11) in 1943, greenhouse tests were carried out in Illinois, using Semesan Jr., Cuprocide, Barbak C, and Spergon. Cuprocide failed to benefit emergence but the other protectants caused a significant average increase in stand.

In Iowa in 1943, Melhus *et al.* (18) investigated the effect of seed treatments on soybeans, the results of which indicated that though the stand was increased by certain materials, the yield remained unaffected.

In 1943, Petty (21) reported that the use of Arasan, Spergon, and New Improved Ceresan in Maryland tests resulted in about 10 per cent increase in stand.

Heuberger and Manns (9, 10), reporting, in 1943, on tests carried out in Delaware, stated that Arasan, Spergon, Ceresan, and Dow No. 5 accelerated emergence of seedlings and increased final stand of plants; Arasan, however, being the only material to increase the yield significantly.

In summarizing his investigations on the influence of fungi on storage, seed viability, and seedling vigor of soybeans, Tervet (26, 27) stated that severe retardation in seedling growth resulted from storage conditions favoring the developing of *Aspergillus spp.*, but seed treatment with the maximum adhesive load of Arasan improved the vigor and stand of plants.

From Puerto Rico, Stoddard (24) reported, in 1944, that treatment of Seminole soybean seed with Arasan, Semesan, and Spergon gave valuable protection against seed rots and pre-emergence damping-off.

Porter (22) and Koehler (14), in 1944, and Allington *et al.* (1), in 1945, reported the results of coordinated, interstate seed-treatment projects involving tests on oil-type and edible soybeans with Spergon, Arasan, Fermate, Semesan Jr., and New Improved Ceresan. While in many instances significant increases in stand were obtained from treatment, nevertheless, in general, yield remained unaffected. Koehler (14) expressed the opinion that tests must be continued over a period of years before definite conclusions can be reached; the same author (15) and Allington and co-workers (1) are doubtful if treatments will be beneficial, or should be recommended, except in a year following a season in which the seed has undergone severe weather damage.

The work of Kiesselbach (12) and of Bartholomew (3) offers no suggestion that significant benefits are to be derived from the treatment of soybean seed with plant hormones.

MATERIALS AND METHODS

Seed of the variety A. K. Harrow, which is grown most extensively in Essex county and which, therefore, was used in the present investigations, was supplied by the Harrow Experimental Station or obtained as required from various commercial sources in the district. Examination of seed and, where necessary, segregation into different categories was done by hand-sorting with the aid of a dissecting microscope. The protectants used were Spergon (tetrachloro-parabenzoquinone), Arasan (tetramethyl thiuramdi-

sulphide), and Fermate (ferric dimethyldithiocarbamate), the first-mentioned at the rate of 3 oz. (0.312 per cent), the two latter at 2 oz. (0.208 per cent) per bu. Treatment was during the first week in February each year, following which the seed was packaged and stored at laboratory temperatures until planting time in May. Each year, prior to spring ploughing, the soil, a Fox sandy loam, received an application of a 2–12–10 commercial fertilizer applied broadcast at the rate of 250 lb. per acre. The addition of a legume inoculant was not considered necessary since crops of soybeans had been recently grown on the site selected for the experiment. All planting was done by hand in a randomized, 5-replicate design, the test unit within each plot comprising duplicate rows, 35 ft. long in 1944, 30 ft. in 1945. The rows were spaced 30 inches apart and, in the seed-treatment experiments, were planted at the rates of 35 and 30 lb. per acre, in 1944 and 1945, respectively.

Periodic examinations, begun a few days after planting and continued throughout the season, furnished the data on emergence and the incidence

TABLE 2.—*Comparison of 1942 poor seed and 1943 good seed*

Year	Percentage			
	Healthy seed	Diseased seed	Broken seed and trash	Germinability
1942	54.29	39.84[a]	5.85	23.4
1943	85.73	2.59	11.68	87.2

[a] This diseased portion comprised the following sub-categories: mildewed or moldy seed, 77.11 per cent; dwarfed and distorted seed, 6.21 per cent; discolored seed coat, 12.42 per cent; wrinkled seed coat, 4.23 per cent.

and severity of disease. In harvesting, the plants were cut near the base of the stem with pruning shears, assembled in bundles, and threshed[4] the same day. All weighings and necessary moisture determinations were made immediately after threshing.

TREATMENT OF DIFFERENT SEED LOTS

Due to extremely unfavorable weather conditions in 1942, soybean seed produced in southwestern Ontario that year was, in general, the poorest for many years, not only being low in viability but also high in disease potentiality.[5] In marked contrast, seed produced in 1943 was of outstandingly high quality. Eight-pound, composite samples of 1942 and 1943 seed, being offered for sale in the district for seeding purposes, were compared as to their germinability and also segregated by hand-sorting into their various component categories with results as shown in table 2.

With seed-lots of two successive years showing such marked differences, the opportunity to test the effect of seed treatment seemed to be an especially

[4] J. L. Jones, No. 4, pea and bean thresher.

[5] This term refers to the capacity of the seed to induce disease in the progeny to which it gives rise.

excellent one. Consequently, on May 15, 1944, treated as well as nontreated seed of the variety A. K. Harrow produced in 1942 were planted at the rate and in accordance with the methods described. Two days later, May 17, the experiment was repeated in exact replica except that the higher quality 1943 seed was used. On May 24, nine days after planting, the seedlings from 1942 seed were breaking ground. On May 29, fourteen days from date of planting, the Spergon-treated rows in all 5 replications were superior to all others not only because of more uniform emergence but also because of outstanding vigor of seedlings. This superiority was readily noticeable until well on into the growing season. In general, seedlings from 1943 seed broke ground earlier and much more uniformly than did those from 1942 seed. On May 29, twelve days after planting, it was apparent that in all 5 replications for 1943 seed Spergon-treated rows were superior to the others, but this superiority was not so marked as in replications for 1942 seed.

TABLE 3.—*Emergence, incidence of disease and abnormality, and yield in relation to difference in quality and treatment of A. K. Harrow 1942 and 1943 soybean seed*

Treatment	Total emergence		Diseased and abnormal seedlings in per cent		Yield in pounds			
					1942		1943	
	1942	1943	1942	1943	Total	Av.	Total	Av.
Fermate	256	1148	21.8	3.9	23.9	5.9	26.3	6.5
Arasan	283	1145	21.2	6.5	22.9	5.7	25.9	6.4
Spergon	712	1280	14.3	1.3	30.9	7.7[a]	25.8	6.4
Check	348	1154	22.4	6.4	24.9	6.2	26.1	6.5
Total	1599	4727	.....	.....	102.6	.....	104.1	.....

[a] Highly significant over Arasan, Fermate, and Check. L.S.D. for averages, 1 per cent = 1.47; 5 per cent = 1.02.

Three weeks after planting, emergence of seedlings and incidence of diseased and abnormal seedlings[6] were recorded, and on October 11 the plots were harvested and yields determined. Data for the two experiments are summarized in table 3.

The germinability of the 1942 seed was extremely low as compared with that of the seed produced in 1943. The nontreated 1942 seed, for example, produced only 348 seedlings, whereas from the 1943 nontreated seed planted at the same rate, 1154 seedlings developed. With both the poor and the good seed, Spergon treatment gave highest emergence of seedlings. Fermate and Arasan, in marked contrast to Spergon, apparently depressed emergence of the 1942 poor seed even below that of the nontreated check. This seemingly deleterious effect was not observed, however, in the case of the 1943 good seed.

[6] Among diseased and abnormal seedlings were included those which, by discoloration or necrosis of hypocotyl, cotyledon, or first-formed pair of true leaves, showed positive evidence of a diseased condition, or which, by distortion or malformation of the above-mentioned parts or by delayed development following slower emergence, differentiated themselves readily from the normally developing seedlings of corresponding age.

The incidence of disease and abnormality was relatively much higher among seedlings originating from the 1942 poor seed than from the 1943 good seed. Spergon-treated seed of both years gave lowest incidence of diseased and abnormal seedlings.

In regard to yield it will be noted that the differences between treatments varied more widely in the case of the 1942 seed than in that of the seed produced in 1943, and that, whereas Spergon treatment gave the highest yield with 1942 seed, Fermate treatment gave the highest yield with 1943 seed. When the data for the two different lots of seeds were subjected to the regular analysis of variance in which the check was considered as a treatment, only the difference in yield arising from the treatment of the 1942 poor seed with Spergon was statistically significant.

It would seem from the data for 1942 poor seed (Table 3) that there is a parallelism, if not a correlation, between emergence and yield. Examining the data recorded for the 1943 good seed, it is to be noted, however, that such a correlation does not exist. For example, emergence from Spergon-treated seed was 9 per cent higher than that from nontreated check, yet yield from the latter was higher. The question of emergence in relation to yield will be dealt with more fully in a later section.

EFFECT OF RATE OF PLANTING ON YIELD AND DISEASE

An experiment was planned in 1944 for the purpose of finding (a) a planting rate that might give optimum yield for the particular type of soil on which the tests were being carried out and (b) if density of plants in the row might be correlated with the incidence, spread, and severity of disease. To avoid disparity in yield that might arise from lack of uniformity in the seed, the latter was selected by hand-sorting from a lot (1943 A. K. Harrow) furnished by the Harrow Experimental Station. This selected healthy seed was planted May 18, at 4 different rates, namely, 20, 30, 40, and 50 lb. per acre. On June 8, three weeks after planting, emergence counts were made, and on October 16, the 5-replicated, randomized plots comprising the experiment were harvested and yield results taken immediately after threshing. Emergence and yield data are summarized in table 4.

TABLE 4.—*Emergence and yield in relation to difference in planting rate of selected healthy A. K. Harrow soybean seed*

Planting rate (lb. per acre)	Emergence	Yield		
		Pounds		Bu. per acre
		Total	Av.	
20	878	25.5	6.3	26.5
30	1304	27.4	6.8	28.5
40	1717	25.5	6.3	26.5
50	2145	24.6	6.1	25.5
.....	6044	103.0	.....	.....

As might be expected, emergence was very closely correlated with rate of planting. It will be noted that (1) the highest yield was obtained from a planting rate of 30 lb. per acre, (2) yields from planting rates of 20 and 40 lb. per acre were equal, and (3) the lowest yield was obtained from a planting rate of 50 lb. per acre. When the data were subjected to the regular analysis of variance it was found that the differences in yield were not statistically significant. The important point, however, is the indication of what an important rôle some factor, probably competition, must play in modifying yield. In table 4, it will be noted that the 1304 seedlings derived from the 30-lb.-per-acre planting rate, yielded 27.4 lb. of seed, whereas the 2145 seedlings derived from the 50-lb.-per-acre rate, yielded only 24.6 lb. It would seem then, that from contiguous plots of ground of equal size and of corresponding soil type, planted with a given lot of seed, yields will be practically equal regardless of relatively wide differences in planting rates. Furthermore, it will be noted that from the 6044 seedlings that developed from the 1943 selected healthy seed, the yield of 103 lb. was not appreciably more—and certainly not significantly more—than the 102.6-lb. yield (See table 3) produced by the 1599 seedlings that originated from the 1942 poor seed. From the foregoing and other examples that the data would provide, it would appear that yield from exactly the same area (approximate 1/15 acre) was only slightly affected by treatment, selection, viability, or disease potentiality of the seed.

TREATMENT OF COMMERCIAL SEED LOTS IN 1945

To confirm previous findings or to modify them, if necessary, a further experiment in seed treatment was conducted in 1945. With few exceptions, soybeans harvested in the fall of 1944 appeared to be as healthy as those of 1943 that had matured under similar favorable conditions, though tests showed their germinability (82.4 per cent) to be slightly below that of the 1943 seed (87.2 per cent). On May 24, seed of the variety A. K. Harrow, produced by the Harrow Experimental Station in 1944, and very fairly representative of commercial seed being offered for sale in the district in the spring of 1945, was planted following treatment with Spergon (3 oz.), Arasan (2 oz.) and Fermate (2 oz.) on the site of the 1944 experiment with the 1942 seed. In the new experiment the length of the rows was reduced to 30 ft. and the rate of planting to 30 lb. per acre. On June 7, fourteen days after planting, the seedlings were breaking ground. The previous year, the seedlings from 1942 seed broke ground in 9 days. The delay in 1945 was due no doubt to the occurrence of a 5-day period of cold, wet weather. As in previous experiments, notes on emergence of seedlings and on incidence of disease and abnormality among seedlings were taken three weeks after planting, and at the end of the season yields were determined. Data acquired from 1944 high-quality seed and 1943 high-quality seed are summarized in table 5. Since length of row and rate of planting are different in the two experiments, results in table 5 are expressed on a percentage basis so that equitable comparison can be made.

Considering first the results obtained with the 1944 seed, table 5 shows that Fermate-, Arasan-, and Spergon-treated seed gave higher emergence than nontreated seed and that there was no significant difference between the effectiveness of the treatments with the three protectants. Incidence of disease and abnormality was highest among seedlings developing from nontreated seed and lowest among those originating from Spergon-treated seed. Fermate-treated seed gave the highest yield with Spergon- and Arasan-treated seed and nontreated seed following in the order mentioned. None of the differences between treatments was found to be statistically significant.

In comparing the results of the two experiments, it will be noted that emergence from 1943 treated and nontreated seed was higher than from similarly treated (or nontreated) 1944 seed. Incidence of disease and abnormality was (a) higher among seedlings from the 1944 seed than from the 1943 seed, (b) highest in both years from nontreated seed, and (c) lowest in

TABLE 5.—*Comparison of results of treatment of high quality commercial soybean seed, variety A. K. Harrow, produced in 1943 and 1944*

Treatment	Percentage				Yield (bu. per acre)	
	Emergence		Incidence of disease and abnormality			
	1943	1944	1943	1944	1943	1944
Fermate	85.4	80.0	3.9	12.0	27.3	27.2
Arasan	85.1	79.6	6.5	13.9	26.8	24.9
Spergon	95.2	78.6	1.3	9.4	26.7	27.1
Check	85.8	62.8	6.4	25.4	27.1	24.9

both years from Spergon-treated seed. It is possible only to surmise as to the reason for the lower emergence of seedlings and the higher incidence of disease among seedlings from the 1944 seed. As pointed out, a 5-day period of cold, wet weather intervened between the planting and emergence of the 1944 seed, whereas in the case of the 1943 seed the weather was continuously favorable and the seedlings broke ground in 9 days. It seems reasonable to suppose that as the result of the 5-day exposure to cold, wet soil, some of the 1944 seeds failed to germinate while others that did germinate were destroyed by pre-emergence damping-off. If unfavorable environmental conditions were the cause of the differences observed, then it appears that under such circumstances the efficacy of seed treatment tends to be appreciably reduced.

In both years it was found that yield from treated seed was in no case significantly higher than from nontreated seed. In both years, too, identical treatments resulted in closely similar yields. This might occasion some surprise because emergence was lower from the 1944 than from the 1943 seed and at the same time incidence of disease and abnormality was appreciably higher from the former than from the latter. These facts not only confirm

but add emphasis to the comments made earlier in the present paper that, though stands of plants may differ widely numerically in the earlier part of the growing season, they tend to yield the same. Thus, in any consideration of factors modifying yield, the ability of the soybean plant to adapt itself to widely-varying degrees of competition must be regarded as important.

TREATMENT OF ETCHED AND CRACKED SEED

As a probable result of the highly favorable weather conditions that pertained in 1943 and 1944, the soybean seed produced in the district in those two years was virtually free from disease. In a critical examination of the 1944 seed (*i.e.*, Harrow Experimental Station A. K. Harrow), it was found, however, that such seed could be separated into different categories, at least two of which, (a) seed with cracked coat, and (b) seed with etched[7] or pitted coat, might, it was thought, have some pathological significance. The seed in question had been harvested with a commercial type, self-propelled

TABLE 6.—*Qualitative and quantitative "analysis" of combined and threshed 1944 A. K. Harrow soybean seed*

Seed harvested by	Categories, in per cent				
	Healthy	Etched or pitted coat	Cracked coat	Diseased	Broken seed and trash
Thresher[a]	78.25	17.44	1.13	0.51	2.65
Combine[b]	62.20	13.26	9.73	0.46	14.31

[a] J. L. Jones, No. 4, pea and bean thresher.
[b] Commercial type, self-propelled combine.

combine. When seed of the same variety that had been grown in the laboratory plots, but which had been threshed with a pea and bean thresher, was examined, it was found that while it, too, showed a relatively high proportion of etched seed, seeds with cracked coats were relatively rare. The evidence suggested that harvesting by combine must have resulted in appreciably heavy seed-coat injury. To investigate the matter further, commercial samples of soybeans (all variety A. K. Harrow) known to have been harvested with a combine were obtained. A cursory examination showed that in every sample, seeds with cracked coats could be readily found. These samples from outside sources together with some of the Harrow Station seed were then thoroughly mixed and from the mixture a 4-pound composite sample was taken. A 4-pound lot was also obtained from the seed produced in the laboratory plots. The two lots were carefully examined under the dissecting microscope and segregated by hand-sorting into the categories shown in table 6.

Two interesting and significant points were: (a) the extremely low and the relatively high percentage of diseased and healthy seed, respectively, in

[7] The term etched seed is used to describe seed that showed lighter colored surface markings which closely resembled the tracery of a filigree pattern and with which coincided a more or less roughened seed coat.

both threshed and combined seed, and (b) the much higher percentage of trash and seeds with cracked coat in the combined than in the threshed seed.

Since in the case of the etched seed as well as in that of the seed with the cracked coat the surface was either roughened or definitely broken and might, therefore, be expected to offer a "lodging-place" for pathogenic organisms, it was thought that these two categories might constitute highly suitable material for a seed-treatment test. Consequently, sufficient etched and cracked-coat seed, as well as healthy seed to serve as check, was obtained to plant duplicate, 30-ft. rows in the randomized, 5-replicate design adopted for all of the experiments. One of the duplicate rows in each replicate was planted with Spergon-treated seed (3 oz. per bu.), the other with nontreated seed. Data as to emergence of seedlings, incidence of disease, and final yield are recorded in table 7.

TABLE 7.—*Results from treated and nontreated components of 1944 commercial A. K. Harrow soybean seed*

Category	Emergence		Incidence of disease and abnormality, in per cent		Average yield (bu. per acre)		
	Spergon	Check	Spergon	Check	Spergon	Check	Difference due to treatment
Etched	570	513	7.5	15.0	27.4	25.5	+1.8
Cracked-coat	529	366	19.6	35.7	25.8	21.5	+4.3[a]
Healthy	695	541	4.7	17.3	26.8	25.1	+1.6

[a] Significant at 5 per cent level.

Treatment with Spergon increased emergence from seeds in all three categories and correspondingly reduced incidence of disease and abnormality. In the case of both treated and nontreated seed, emergence was highest from healthy seed, intermediate from etched seed, and lowest from cracked-coat seed; whereas incidence of disease and abnormality was lowest for healthy seed, intermediate for etched seed, and highest for cracked-coat seed. Furthermore, treatment with Spergon increased the yield in the case of each component. When, however, the difference between the two means of each component was compared by the t test (8), only that between treated and nontreated, cracked-coat seed was found to be statistically significant. Since the seeds in this category were the most doubtful from the standpoint of germinability and seemingly the most likely to harbor pathogenic organisms, the results indicating an increase of 4.3 bu. per acre due to treatment with Spergon are regarded as being of considerable importance. In southwestern Ontario, most soybeans are harvested with combine. The latter are not always carefully adjusted and operated and as a result an appreciable fraction of the crop may be liable to injury, the degree and amount of which will probably vary with the moisture content of the soybeans at time of harvest.

The results of this experiment furnish an additional example of the lack of correlation between number of plants in early stands and final yield. Referring to table 7, it will be noted that the emergence of Spergon-treated healthy seed exceeded that of nontreated healthy seed by 154 seedlings, yet the difference in yield from identically the same lengths of row, planted originally with the same numbers of seeds, was not statistically significant.

INCIDENCE OF BROWN SPOT IN DIFFERENT VARIETIES IN 1945

The rapidly expanding acreage of soybeans in southwestern Ontario has accelerated the introduction of new varieties and selections. It was felt that before these should become established information should be obtained as to their susceptibility or resistance to the diseases prevalent in the district. With this in view, seed of the varieties Richland, Lincoln, Earlyana, and Harman, and of a Harrow Experimental Station selection, designated as Harrow A,[8] were planted in a 5-replicate, randomized design on May 25, 1945. The plots were kept under critical observation throughout the grow-

TABLE 8.—*Relative incidence of brown spot infection on different varieties of soybeans*

Varieties	Number of plants examined	Percentage of infected plants
Earlyana	633	89.5
Lincoln	682	67.7
Richland	432	66.6
Harman	562	39.6
Harrow A	349	37.5

ing season. With the exception of brown spot caused by *Septoria glycines*, the plots remained virtually free from disease. Brown spot was first noted in the varietal plots on July 3. The typical reddish brown spots were especially noticeable on the pair of unifoliate or first true leaves. Casual examination of the different varieties suggested differences in susceptibility and resistance. Counts of the unifoliate leaves with infection on July 7 are recorded in table 8.

On a basis of their relative susceptibility to brown spot, the varieties Harrow A and Harman would be classed as moderately resistant, Richland and Lincoln intermediate in susceptibility, and Earlyana highly susceptible. Not only were more leaves of the variety Earlyana infected than those of the other varieties, but they were more severely infected. Even on Earlyana, however, infection spread beyond the first pair of unifoliate leaves to only a few of the lower trifoliate leaves.

The unifoliate leaves of the variety A. K. Harrow, originating from seed treated with Arasan, Fermate, and Spergon, respectively, were no less seriously infected than those of plants developing from nontreated seed, thus

[8] For seed of this selection and of the varieties mentioned, the writers are indebted to Mr. C. Owen, Dominion Experimental Station, Harrow, Ontario.

indicating lack of correlation between incidence of brown spot and seed treatment.

DISCUSSION

The results obtained in the present investigations are in close agreement with those reported more recently by several workers, including Melhus *et al.* (18), Porter (22), Koehler (14), and Allington *et al.* (1), all of whom have found that although seed treatment might give increased stands over non-treated checks, in general, yields remained unaffected. These investigators for the most part doubt that seed treatment can be recommended except in a year following a season in which the seed has undergone severe weather damage, or when, for some reason, it might be desired to "stretch" seed by sowing less than the amount required under normal circumstances. With the above, the present writers are in accord except that with the evidence of their own experiments to lend added weight, they would express themselves more positively as to the advisability of treating the poor seed that is produced in "bad" years. They also feel that when, as the result of being harvested with a combine either unsuitable as to type or not carefully enough operated or adjusted in relation especially to existing weather conditions (too many instances of which have been encountered in Ontario), soybeans have an appreciably high percentage of seeds with a cracked coat, benefits will accrue from seed treatment. The situation over the next few years would seem to resolve itself into one of educating the grower to the point where by visual inspection he can decide for himself whether seed should be treated or not.

Of interest in the present investigations were the repeated demonstrations of remarkable uniformity of yield from plots of equal size despite wide differences in the number of plants involved. For example, it was shown that from the initial stand of seedlings from 1942 poor seed the yield was not appreciably less than that produced by almost four times as many seedlings originating from specially selected healthy 1943 seed in exactly the same length of row. Other examples similar to the above were encountered, and, considering them altogether, they provided substantiation for the statement made earlier in the present paper to the effect that, within reasonable limits, yields from contiguous plots of ground will tend to be affected only slightly by rate of planting, treatment, selection, germinability, or disease potentiality of the seed. Indeed, a new appreciation was gained not only of the capability of the soybean plant to adapt itself and respond to widely varying degrees of competition but also of the importance of this latter factor in relation to yield. With other than seed of very low germinability or of high disease potentiality, it would seem that optimum planting rate—with due consideration for soil type—might be as important as almost any other factor in obtaining maximum yield. It is noteworthy that Probst (23) who recently studied the effect of plant spacing of soybeans on yield, reported that "the least difference in yield, and usually the highest yield, was obtained

when the plants were spaced 2 or 3 inches apart." Certainly, in seed treatment experiments with soybeans, increase in early stand of plants as the result of treatment should not be regarded as necessarily indicating corresponding increase in yield.

SUMMARY

In a 3-year series of experiments (randomized, 5-replicate design), the efficacy of Spergon, Arasan, and Fermate has been tested on seed lots of the variety A. K. Harrow differing widely as to germinability, disease potentiality, and extent of seed-coat injury. With poor quality, weather-damaged seed, such as that produced in 1942, and with the cracked-coat fraction (resulting from combine injury) of an otherwise high-quality seed, such as that produced in 1944, treatment with Spergon increased emergence and yield. These were the only instances, however, in which increases in early stands of plants as the result of seed treatment were correlated with statistically significant increases in yield.

When selected healthy seed was planted at the rates of 20, 30, 40, and 50 lb. per acre, emergence of seedlings was closely correlated with rate of planting but differences in yield were not statistically significant. The results of this and other tests having shown a complete lack of correlation between stands of plants, differing numerically by a ratio almost as high as 4:1, and yield, it was concluded that the increase in early stand of plants as the result of treatment should not be regarded as necessarily indicating a corresponding increase in yield.

In a varietal resistance test, Earlyana was most susceptible, Lincoln and Richland were intermediate in susceptibility, and Harman and Harrow A (a promising unnamed selection) were most resistant to brown spot caused by *Septoria glycines* Hemmi, but the incidence of brown spot was in no way correlated with either treated or nontreated seed.

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ORGANIC COMPOUNDS FOR CONTROL OF TOBACCO BLUE MOLD

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INTRODUCTION

The blue-mold disease of tobacco (*Nicotiana tabacum* L.) caused by *Peronospora tabacina* Adam is of major importance in the tobacco growing areas of the United States, particularly in the southeastern states of Georgia, North and South Carolina, and Virginia. The disease is more severe in cool, moist weather, which frequently occurs in the early spring months when seedling tobacco plants are small and succulent. This coincidence is favorable to epidemic outbreaks which have recurred each year since 1931, with consequent delay in transplanting, and often serious plant shortages. The continued destructiveness of blue mold has stimulated much research on methods of control by several State and Federal agencies. The two treatments that were first developed and introduced commercially were copper oxide–oil spray (2) and fumigation treatment with paradichlorobenzene (3). Further investigation showed that organic fungicide sprays and dusts were effective also, particularly certain of the salicylate compounds as well as Fermate and other organic fungicides (1, 4, 7, 8).

Tobacco growers generally have not adopted these control measures, but instead have depended on increased yardage of plant beds and other cultural practices. Such methods have not been effective under epidemic conditions of blue mold, as was clearly demonstrated in North Carolina in 1945, when over 90 per cent of the crop of some entire counties was set 10 to 20 days later than normal or was set from plants transported as far as 200 miles. Therefore, the need exists for an effective treatment which is easy to apply and inexpensive enough to adopt as a part of the general operations of the grower. This paper reports further results in the continuing search for less expensive fungicides, more efficient formulations, and more convenient methods of application.

EXPERIMENTAL METHODS

Both greenhouse and outside plant-bed tests were used in evaluating the fungicides. Results obtained in the greenhouse were regarded as preliminary, inasmuch as plant-bed conditions could not be fully duplicated. Blue mold was easily maintained and very active in the greenhouse during the winter months. Unprotected check plants usually had 60 to 100 per cent of leaf area killed.

The disease in plant beds was more severe in cool, cloudy, or rainy

[1] Cooperative investigations by the Division of Tobacco, Medicinal and Special Crops, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, the Coastal Plain Experiment Station of Georgia, the South Carolina Agricultural Experiment Station, the North Carolina Agricultural Experiment Station, and the North Carolina Department of Agriculture.

weather, and these conditions were frequent enough during the seasons of 1940, 1941, 1943, 1944, 1945, and 1946 to give critical tests of the fungicides used. Conditions were unfavorable for the development of blue mold during 1942, and the results were not regarded as critical. These data are not included.

Plants were grown in 4- or 6-inch pots for greenhouse experiments. Uniform stands of 6 to 12 plants were used in 4-inch pots, and 15 to 20 plants in 6-inch pots. Treatments were begun on plants four to six weeks old, when leaves were $\frac{1}{2}$ to $\frac{3}{4}$ inch in diameter. Two to three pots were used in preliminary experiments, and five to ten pots in critical tests. Sprays and dusts were applied twice weekly with a small hand sprayer or duster during a period of 3 to 4 weeks. Plants were inoculated after the third or fourth treatment and placed under a moist muslin tent.[2] Disease severity was determined 1 to 3 weeks after the final treatment, by counts of the dead and partly killed leaves. Injured leaves were placed in 3 classes of disease severity, *i.e.*, entire leaf area dead, approximately one-half of leaf area dead, and approximately one-fourth of leaf area dead. Percentage of leaves in each class was determined from total number of leaves exposed to infection. The percentages in class 1 were multiplied by 1.0, those in class 2 by 0.5, and those in class 3 by 0.25; and the sum of these values was used as the disease index in which 0 indicates no symptoms and 100 complete defoliation.

Plant-bed treatments were started when tobacco leaves were one-half to one inch in diameter. Plants usually reach this stage the last week in March, or the first week in April, at the Experiment Station in Florence, South Carolina. Dusts and sprays were applied twice weekly, which was sufficient to keep new growth covered with fungicide. Plants were inoculated after the third or fourth treatment, and also were subject to natural infection by wind-blown spores from adjacent untreated beds. Disease readings were taken 1 to 3 weeks following final treatment.

EXPERIMENTS WITH SPRAYS

From 1940 through 1946 numerous spray and dust experiments were completed at the Florence, South Carolina, Experiment Station. More than 250 materials were tested in the greenhouse in the course of approximately 75 separate spray or dust experiments. Plant-bed tests also were conducted each year in which all materials were included that showed promise in the greenhouse. In addition to this, there were extensive supplementary plant-bed tests each year at the Georgia Coastal Plain Experiment Station, at Tifton, Georgia, and at the Tobacco Branch Experiment Stations at Oxford and at McCullers, North Carolina. A large mass of experimental data was obtained, obviously much too detailed for complete presentation here. Results are therefore given only from the most significant or the most illus-

[2] A muslin tent was placed over a portion of the greenhouse bench, above which a spray nozzle was suspended. Relative humidity was 100 per cent when spray was running. Inoculated plants were kept under the tent for 1 to 2 weeks to allow for infection and abundant sporulation by the blue-mold fungus.

trative experiments. The salicylate compounds proved to be particularly effective. Clayton *et al.* (4) reported the value of bismuth subsalicylate and benzyl salicylate, the latter used with cottonseed oil. Brief reports were also published by the senior writer (7, 8) on the effect of various salicylates and certain other organics used in both non-oil sprays and in dust preparations. In the earlier greenhouse and plant-bed tests copper oxide–oil was used for comparison. The degree of protection given by some of the salicylates used without oil was higher than that for copper oxide–oil. Materials were dissolved or suspended in suitable solvents from which dilutions were made with

TABLE 1.—*Blue-mold control in the greenhouse with salicylate sprays*

Material	Amt. per 100 gal. of spray	Disease index[a]				
		Plot 1	Plot 2	Plot 3	Plot 4	Average
	Lb.					
Acetylsalicylic acid	0.25	8	5	8	13	8.5*[b]
Benzyl salicylate	1.0	18	7	17	12	13.5
Betanaphthyl salicylate	0.25	11	10	11	18	12.5
Bismuth subsalicylate	1.5	0	0	0	0	0 *
Copper oxide–oil	1.0–1.0[c]	22	25	17	12	19.0
Isoamyl salicylate	1.0	11	7	13	5	9.0*
Methyl salicylate	1.0	24	25	37	30	29.0
N-butyl salicylate	0.5	23	6	10	15	13.5
Phenyl salicylate	0.5	30	33	38	28	32.2
Salicylaldehyde	1.0	16	15	36	27	23.5
Salicylamide	1.5	20	8	9	15	13.0
Salicylic acid	0.5	6	5	13	15	9.7*
Sodium salicylate	0.5	17	12	24	15	17.0
Zinc salicylate	0.25	25	30	27	23	26.2
Control (not treated)	.....	87	95	72	78	83.0
Difference required for significance at 5 per cent level						8.25
Difference required for significance at 1 per cent level						11.02

[a] The disease index used here is a percentage figure, which is the sum of the disease values assigned to leaves in 3 classes of blue mold severity; *i.e.*, percentage with entire leaf area dead × 1, plus percentage with ½ of leaf area dead × 0.5, plus percentage with ¼ of leaf area dead × 0.25. 100 equals complete defoliation and 0 equals no symptom.

[b] Values significantly lower than the value for copper oxide–oil at 5 per cent level are indicated by an asterisk (*).

[c] One lb. copper oxide and 1 gal. cottonseed oil for 100 gal. of spray.

water after emulsifying with 0.01 per cent B–1956 emulsifier.[3] Some 24 salicylates have been tested.

Table 1 presents results from a typical greenhouse experiment in which 13 salicylates were included. Bismuth subsalicylate was outstanding, and has been consistently so in both greenhouse and plant beds. (Fig. 1.) Good results were obtained also with acetylsalicylic acid, isoamyl salicylate, and salicylic acid. Each was more effective than copper oxide–oil. The margin between fungicidal and phytotoxic levels was relatively narrow with salicylic acid, acetylsalicylic acid, and betanaphthyl salicylate. Other salicylates tested but not listed in table 1 were: ethyl salicylate, isopropyl salicylate, salicylaldoxime, sodium hydrogen sulphosalicylate, and sulphosalicylic acid,

[3] A phthalic anhydride glycerol alkyl resin.

5-aminosalicylic acid hydrochloride, 3,5-dinitrosalicylic acid, salicylideneacetamide, and thiosalicylic acid. This latter group gave varying degrees of slight to moderate protection from blue mold. None gave such consistently high protection as to justify continued tests with them. While results in table 1 indicate the fungicidal value of the respective salicylates, these and other greenhouse results were not always in close agreement with those obtained in plant beds. Results here, therefore, are not intended to imply the value of these materials in plant beds. For example, in plant-bed tests at Tifton, Georgia, bismuth subsalicylate and zinc and benzyl salicylates were rated highest, and acetylsalicylic acid was somewhat less effective than indicated in table 1.

FIG. 1. Control of blue mold with salicylate sprays. Vertical rows, left to right: untreated, disease index 83; zinc salicylate, disease index 26; bismuth subsalicylate, disease index 0; betanaphthyl salicylate, disease index 13; benzyl salicylate, disease index 14.

Investigations by workers in recent years demonstrated the fungicidal value of numerous recently synthesized organic compounds. Tisdale and Williams (11) originally described the fungicidal properties of certain derivatives of dithiocarbamic acid. One of these, Fermate, (ferric dimethyldithiocarbamate) has since been proven effective by various workers (12). Disodium ethylene bisdithiocarbamate (Dithane) is another derivative which is water-soluble and highly fungicidal (5). Other derivatives include zinc dimethyldithiocarbamate (Zerlate) and tetramethyl thiuramdisulphide (Thiosan and Arasan). The latter is used as a seed and turf fungicide. Tetrachloro-parabenzoquinone (Spergon) is chemically unrelated to the above compounds, but is widely used as a seed protectant. The following are other recently studied organics that have been proven fungicidal: 2,3-dichloro-1,4-naphthoquinone (U.S.R. No. 604), 2,2-dihydroxy-5,5 dichloro-

phenylmethane (Preventol GD), and phenanthra quinone. All of these and numerous others were used against blue mold. The great majority were not effective, some gave slight to moderate protection, and a few were very effective.

Table 2 presents a summary of results from greenhouse and plant-bed

TABLE 2.—*Amount of blue mold in greenhouse and plant-bed tests of organic and other spray materials, 1941–45*

Material	Greenhouse			Plant beds		
	Amt. per 100 gal.	No. of tests	Average disease index	Amt. per 100 gal.	No. of tests	Average disease index
	Lb.			*Lb.*		
Bismuth subsalicylate	1.5	1	1	1.5	10	4
Benzyl salicylate	1.0	1	9	2.0	1	2
Benzoyl peroxide	1.0[a]	4	7	3.0	1	0
Dithane (H.E. 175)	1.0	1	2	2.0	3	3
Copper oxide–oil	1.0	33	19	1.0	24	11
Fermate	4.0	2	0	4.0	5	8
Salicylic acid	0.5	2	10	....[b]	....	....
Zinc salicylate	1.0	5	5	0.25	2	12
Benzoic acid	1.0	2	27	2.0	1	0
Dithane (D–14)	....[c]	1	4	....	1	8
Spergon	1.5	1	6	4.0	3	8
Thiosan	4.0	2	1	4.0	3	50
p-Toluenesulphonylamide	1.0	2	12	1.0	1	41
Diphenyl-pp′-disulphonic acid	1.0	1	8	1.0	1	48
p-Hydroxyazobenzene	0.25	1	6	0.25	1	35
Guaiacol benzoate	2.0	1	2	1.5	3	24
U.S.R. No. 604	1.0	1	0	2.0	1	54
Zinc dimethyldithiocarbamate	1.0	1	2	2.0	1	23
4-Chlorophenyl-4-toluene sulphate	1.5	1	9	....	....	....
2,5-Diaminotoluene sulphate	0.125	1	13	....	....	....
o-Phthallic acid	0.5	1	13	....	....	....
p-Cresyl benzoate	3.0	1	15	....	....	....
Pentachlorophenol	0.0625	1	17	....	....	....
Benzoyl chloride	1.0	2	23	....	....	....
Diethylanalin	3.0	1	25	....	....	....
Isoamyl benzoate	3.0	1	25	....	....	....
Control (not treated)	....	31	78	....	26	60

[a] Only those formulations that gave best control are listed. Benzoyl peroxide, and some of the other materials, were used in varying rates only slightly lower or higher than those listed, so that the total number of tests was greater than that shown in the table, but in each case the final disease index was only slightly different. Benzoyl peroxide is a strong oxidizing agent and is potentially explosive.

[b] No plant-bed results were obtained at Florence, S. C., but in tests at Tifton, Ga., salicylic acid was about equal to zinc salicylate.

[c] According to directions of manufacturer, 2 quarts per 100 gallons, plus 1 lb. zinc sulphate and ½ lb. hydrated lime.

experiments with some of the best organic and other materials during 1941 through 1945. Materials were placed in three groups according to the degree of blue-mold control in plant beds as follows: (1) equal to bismuth subsalicylate; (2) equal to copper oxide–oil; (3) less effective than copper oxide–oil, but with some fungicidal value. Those approaching the control value

of bismuth subsalicylate were most effective. Benzyl salicylate was most extensively tested and gave consistently good results. Benzoyl peroxide and Dithane (H.E. 175) are rated high in table 2, but results were from limited plant-bed tests and these materials have not been sufficiently tested on a large scale to rate them among the best. Those materials in group 2 which gave approximately the same control as copper oxide–oil were Fermate, salicylic acid, and zinc salicylate. Fermate was most exhaustively tested and proved effective under varying conditions. Zinc salicylate was most useful in combinations with other fungicides as described later in experiments with dust materials. A similar effect was found in sprays when zinc salicylate and salicylic acid were combined with Fermate and with Thiosan. A full report of the results with spray combinations will be presented in later publications. Dithane D–14, Spergon, and Thiosan were placed in group 3 of table 2 because they were not consistently effective, although in some tests they appeared equal to Fermate and the best salicylates. Benzoic

TABLE 3.—*Amount of blue mold in tests of selected organic sprays in a plant-bed experiment at Florence, South Carolina, in 1945*

Material	Amt. per 100 gal. of spray	Disease index				
		Plot 1	Plot 2	Plot 3	Plot 4	Average
	Lb.					
Bismuth subsalicylate	1.5	7	6	6	7	6.5
U.S.R. No. 604	1.5	62	61	48	45	54.0
Dithane D–14	[a]	9	6	7	8	7.5
Fermate	4.0	4	6	11	7	7.0
Zinc dimethyldithiocarbamate	2.0	20	16	21	37	23.5
Control (not treated)	.....	90	77	77	70	78.5

[a] According to directions of manufacturer. See table 2, footnote c.

acid, although effective at Florence, S. C., was not equal to the salicylates in tests at Tifton, Georgia. The remaining materials in table 2 were slightly to moderately active as fungicides, but were not so effective as the above. Most of those in group 3 had narrow margins between fungicidal and phytotoxic levels, and results with them did not seem to justify further exhaustive tests. Some of these were tried only in the greenhouse. Table 2 shows that while greenhouse results were usually indicative of the fungicidal value of the materials under test, the results were frequently misleading. Thiosan, zinc dimethyldithiocarbamate, and U.S.R. No. 604 gave good protection in the greenhouse, but failed to do so in plant beds. On the other hand, copper oxide–oil gave slightly better protection in plant beds than in the greenhouse.

Table 3 presents results from a comparative plant-bed test made in 1945 with a group of the better organic sprays. Spray materials, except Dithane D–14, were used with Vatsol OTC as a wetting agent at the rate of ½ pound to 100 gallons of water. Sprays were applied twice weekly during March 19 to April 13. Disease readings were taken March 30. Bismuth subsalicylate, Fermate, and Dithane D–14 were about equally effective; each gave excellent

control. Zinc dimethyldithiocarbamate gave fair control, but U.S.R. No. 604 was relatively ineffective.

Results from spray tests and from observation among growers indicated that certain blue-mold fungicides or combinations have pronounced residual effects. For example, many growers controlled blue mold until the end of the season and later had an outbreak after treatments were stopped, which could be attributed to missing the critical applications or stopping treatments too early. Experience showed that bismuth subsalicylate, for example, could be used with more latitude in treating schedules than Fermate. It has been rather difficult to secure exact data on residual protection, but the following results from the McCullers, N. C., tobacco substation illustrate the residual protection offered by bismuth subsalicylate in a series of cooperative tests among tobacco growers in 1945. Table 4 shows that the three fungi-

TABLE 4.—*Residual effect of bismuth subsalicylate spray on control of blue mold in plant-bed tests, 1945*[a]

Material	Amt. in 100 gal.	Average disease index		
		Days between last treatment and disease readings		
		0–2	7–10	14–16
	Lb.			
Copper oxide–oil	1.0	11.9	7.4	35.8
Fermate	2.0	2.7	3.8	27.9
Bismuth subsalicylate	1.5	0.6	6.6	19.4
Control	.....	67.1	56.3	44.7

[a] Four to 9 spray applications were made in an approximate twice-weekly schedule. Results were summarized from tests at 10 bed locations in Wake County, N. C. In most locations 100 sq. yd. of bed area were treated with each fungicide.

cides compared gave about equal protection for 10 days after sprays were stopped. The extended protection of bismuth subsalicylate was most evident when 14–16 days elapsed, as disease indices were 19.4 for this material, as compared with 27.9 and 35.8, respectively, where Fermate and copper oxide–oil were used. These results were confirmed by observation of numerous plant-bed trials during 1941 through 1945 in Georgia and North and South Carolina.

EXPERIMENTS WITH DUSTS

Tests with dusts, started in 1942 at the Pee Dee Experiment Station, Florence, South Carolina, and later at Tifton, Georgia, and Oxford, North Carolina, have shown that some dusts are effective against blue mold. Copper and sulphur compounds in dust preparations were of little or no value, and among the numerous materials tried only the organics gave good protection. Results in table 5 represent the relative control value of various dusting materials. Bismuth subsalicylate was outstanding and gave excellent protection in repeated tests. Fermate gave good control, but was somewhat less

TABLE 5.—*Amount of blue mold in greenhouse and plant-bed tests of organic dust fungicides, 1943–45*

Material[a]	Amt. per 100 lb. of dust	Greenhouse		Plant beds	
		No. of tests	Average disease index	No. of tests	Average disease index
	Lb.				
Bismuth subsalicylate	10 to 15	6	9	2	4
Dithane (H.E. 175)	5 to 10	5	3	1	5
Fermate	10 to 15	5	13	3	10
Sodium salicylate	0.5 to 1	3	12	2	11
Spergon	10	3	19	1	41
Thiosan	10	1	12	1	46
Zinc salicylate	2	4	16	1	16
Control (not treated)	.....	10	84	4	52

[a] Pyrophyllite, Pyrax ABB, was used throughout as a diluent.

effective than bismuth subsalicylate (Fig. 2).[4] Results with Thiosan and Spergon were erratic, although in some tests they were effective; generally results with them were similar to those obtained in spray tests. Zinc salicylate and sodium salicylate, while effective, frequently caused plant injury. Dithane (H.E. 175) was very effective, but tests with this material were discontinued in 1945 because it was no longer available in a form suitable for dusting.[5]

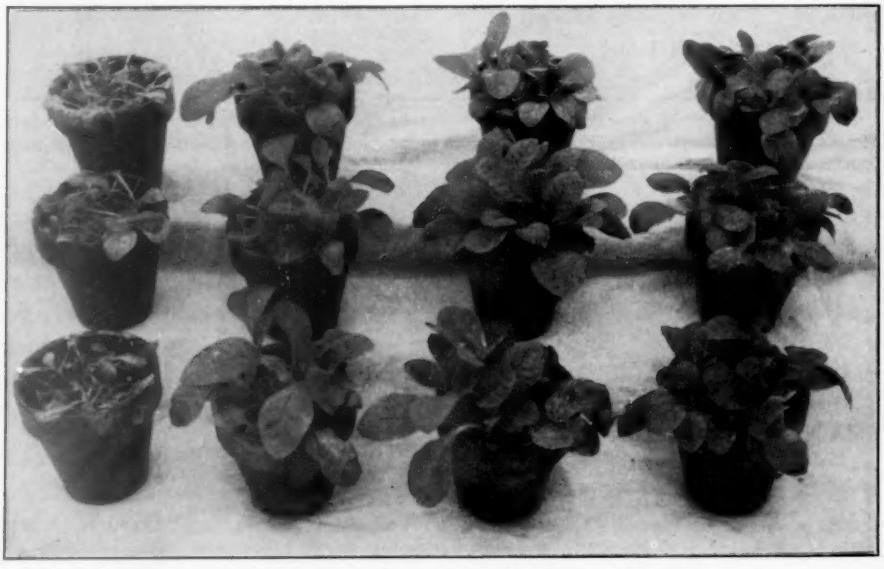

FIG. 2. Control of blue mold with organic dusts. Vertical rows, left to right: Untreated, disease index 92; 2 per cent U.S.R. No. 604, disease index 26; 15 per cent Fermate, disease index 6; 10 per cent bismuth subsalicylate, disease index 0.

[4] In another comparative trial in 1946 bismuth subsalicylate was somewhat less effective than Fermate, while in all other tests the former was consistently best. This reversal of results may have been due to the special grade of bismuth subsalicylate used in 1946; however, this point has not been determined.

[5] The manufacture of this material in dry form was discontinued by the manufacturer, and was replaced by Dithane D–14, a liquid for use in sprays.

In early experiments with single materials the most effective concentrations were found. Tests with Fermate showed that 15 per cent was most satisfactory and that amounts of Fermate above this were not correspondingly better. For example, in one experiment 30 per cent Fermate was no better than 15 per cent. Repeated tests in plant beds confirmed this, and showed that when adequate amounts of dust were applied, 15 per cent gave adequate protection (Fig. 3). Similarly, bismuth subsalicylate, Spergon and Thiosan were most effective in 10 per cent dusts. Zinc salicylate gave maximum protection at 2 per cent, but higher concentrations caused marked plant injury.

FIG. 3. Blue mold in tobacco plant beds. A. Plants protected with Fermate–zinc salicylate dust. B. Untreated plants with severe blue-mold infection.

Table 6 presents results from a plant-bed experiment to determine the amount of dust necessary to control blue mold. Results with Fermate (15 per cent) in a test at Oxford, N. C., in 1944, showed that relatively large amounts and thorough coverage were necessary. Beds receiving 17.25 or more lb. per 100 sq. yd. developed very little blue mold and the bed receiving 11.50 lb. also remained relatively disease-free, but half this amount (5.75 lb.) was not effective. These results are in general agreement with those obtained at Florence, S. C., and at Tifton, Ga., where rates of 16 to 20 lb. per 100 sq. yd. gave adequate control.

Early experiments with dusts indicated that certain dust mixtures gave superior protection against blue mold, an effect that has been described as

TABLE 6.—*Rate of application of Fermate dust in relation to blue-mold control in plant beds*[a] *at Oxford, N. C., in 1944*

Amount of dust used on 100 sq. yd.				Disease index
Treatments 1 and 2	Treatment 3	Treatments 4, 5, 6, and 7	Total for 7 treatments	
Lb.	*Lb.*	*Lb.*	*Lb.*	
0.0	0.0	0.0	0.0	56.5
0.5	0.75	1.0	5.75	33.4
1.0	1.50	2.0	11.50	8.8
1.5	2.25	3.0	17.25	6.5
Fermate dust, not weighed but dusted heavily to give thorough coverage. Seven treatments applied				2.2

[a] Plots were 100 sq. yd. (5×20), and so arranged that one long side of each was adjacent to an untreated area. Applications were made on single plots for each rate with a Root hand-crank duster and applied in a twice-weekly schedule for a total of 7 applications. Dust formulation was 15 per cent Fermate in Pyrax ABB clay diluent.

synergism.[6] This phenomenon was observed by Clayton *et al.* (4) in copper oxide–oil spray used against blue mold. They state, "It is readily possible to show by computation that the protection provided by the combination of copper and oil is significantly greater than the sum of the protection provided by each separately." Dimond and Horsfall (6) observed a similar effect with combinations of copper and sulphur used to control potato tip burn.

The action of dust combinations against blue mold is illustrated by the following greenhouse experiment, which is representative of a series of such tests made during 1944 and 1945. A randomized block test with six single-pot replicates was used. Dusts were applied twice weekly for a total of 6 treatments. Zinc salicylate was combined with Fermate and each of these components also was applied separately in the same concentration. The concentrations used were well below those known to give effective blue-mold control. Dust applications were very light and uniform, and conditions for blue-mold development were very favorable. Table 7 shows that 1.0 per cent

TABLE 7.—*Synergistic effect of a combination of Fermate and zinc salicylate dust on control of blue mold, 1944*

Material	Amount per 100 lb. dust (lb.)	Average disease index
Fermate	1.0	98.8
Zinc salicylate	0.5	83.0
Fermate and zinc salicylate	1.0 and 0.5	45.1
Fermate	15.0	36.8
Fermate	30.0	30.6
Difference required for significance at 5 per cent level		7.8
Difference required for significance at 1 per cent level		10.64

[6] The term synergism is used here to describe the phenomenon in which the total fungicidal effect of combined materials is greater than that of the sum of the component parts used independently.

Fermate reduced blue mold only 1.2 per cent and that 0.5 per cent zinc salicylate reduced it 17.0 per cent. A mixture of the two in the same concentration reduced blue mold 54.9 per cent, which was 36.7 per cent more than the sum of values for the single components. Both 15 per cent and 30 per cent Fermate gave only partial control (disease indices were 36.8 and 30.6, respectively) which shows that light dusting and favorable conditions for blue mold gave a critical test of Fermate and zinc salicylate at the low concentrations used.

Table 8 presents results from further experiments in plant beds during 1945 to obtain more information on the action of combined dusts. A randomized block test was arranged with 4 replicate plots, each of approximately 2 sq. yd. Treatments were applied with a small, hand, plunger-type duster in a twice-weekly schedule during March 20 to April 5. Zinc salicylate was combined with Fermate, Thiosan, and Spergon. Results demonstrated the

TABLE 8.—*Synergistic and residual effect of single and combined dusts on control of blue mold, 1945*

Material	Amt. in 100 lb. of dust	Average disease index when used	
		Alone	Combined with 2 lb. zinc salicylate per 100 lb. of dust
	Lb.		
Bismuth subsalicylate	10.0	7.2	.....
Zinc salicylate	2.0	16.0	.....
Fermate	15.0	19.2	8.0
Fermate	30.0	21.0	.....
Spergon	10.0	41.7	29.2
Thiosan	10.0	46.2	8.7
Control (not treated)	.....	52.7	.....

Difference required for significance at 5 per cent level: 9.47
Difference required for significance at 1 per cent level: 12.79

superior value of the combined fungicides as compared with the single materials. For example, when 2 per cent zinc salicylate was added to 15 per cent Fermate, 10 per cent Thiosan, or to 10 per cent Spergon, disease indices were respectively 8.0, 8.7, and 29.2, but when used without zinc salicylate indices were 19.2, 46.2, and 41.7, in respective order. Differences were significant in each case.

The results in table 8 also show the residual protection of these mixtures, since 21 days elapsed between the final treatment and the disease readings. During the period of treatment (March 20 to April 5) weather conditions were not favorable for active blue mold. However, beginning on April 18 a period of rainy and cloudy weather allowed blue mold to become active so that disease damage could be noted a week later. Results, therefore, are an expression of the residual protection. Bismuth subsalicylate used alone was an outstanding protectant. In earlier tests this fungicide had a marked residual effect in sprays also. The residual protection of the zinc salicylate–

Fermate mixture and the zinc salicylate–Thiosan mixture was approximately equal to that of bismuth subsalicylate. The zinc salicylate–Spergon mixture was comparatively ineffective, although it was much superior to Spergon alone.

TESTS WITH GROWERS

Further information on the practical value of dusts against blue mold was obtained in cooperative tests with tobacco growers in 1945. Several of the growers used 15 per cent Fermate in comparative tests with zinc salicylate combined with Fermate, with Spergon, and with Thiosan (Table 8). Disease control was good except at one location where severe infection had developed before treatments were started. At this location severe blue mold appeared the morning after the first treatment was applied. This indicated infection had become so general in the bed that the treatments had no opportunity to check spread of the disease. It is known that blue mold requires 6 to 10 days for sporulation to occur after plants are inoculated. In all other locations the fungicidal value was increased when zinc salicylate was added to Fermate, Thiosan, or Spergon. Fermate alone, however, gave adequate control, because four growers who used it checked blue mold completely and the remaining five growers found very slight infection that caused practically no damage. In every community where dusts were used, the beds not treated suffered severely from blue mold. In seven of the cooperative dusting experiments, treatments were begun after primary infection had appeared, yet in each case blue mold was effectively checked so that little or no damage resulted. Similarly at the Pee Dee Experiment Station, Florence, S. C., dust treatments were begun on three beds after primary areas of infection started. Secondary spread of blue mold was checked almost immediately, and the primary infections became inactive.

During 1946 a severe epidemic of blue mold in South Carolina gave an opportunity for critical tests with Fermate (15 per cent in pyrophyllite) which was sold commercially and used by many growers.[7] The writer had an opportunity to observe closely the beds of about 15 growers who used Fermate dust. Results demonstrated excellent control of blue mold. Most treated beds remained entirely free of the disease and none of them was delayed in transplanting, while nontreated beds often suffered high plant mortality (50 to 75 per cent) and there was serious delay in transplanting. Reports generally throughout the State and elsewhere have confirmed these observations.

DISCUSSION

Treatment for the control of tobacco blue mold has been simplified and made more effective through the development of organic fungicides for either spraying or dusting. Most of the organic sprays are easily mixed with water and applied directly—a simpler procedure than mixing copper oxide and emulsifying cottonseed oil separately before combining the two. Dust treat-

[7] Approximately 20 tons of Fermate dust were used by South Carolina tobacco growers in 1946.

ments are even more advantageous, as tobacco beds are frequently located so that transporting water to them is a problem.

The salicylate sprays were highly effective against blue mold. Bismuth subsalicylate and benzyl salicylate were tested extensively in commercial beds with most satisfactory results. Salicylic acid and zinc salicylate were somewhat less satisfactory, but were fungicidal when used alone and especially when used in combination with certain sprays and dusts. In addition, five other salicylates probably deserve further testing as fungicides. Fermate spray also has been exhaustively tested by growers, and its value against blue mold is well established, although it is slightly less effective than some of the salicylates. Certain derivatives of benzoic acid gave considerable protection. Benzoyl peroxide was of particular interest, as repeated trials gave good results. It appears unlikely that such a strong oxidizing agent would remain stable long enough to react directly to stop spore germination or initial infection. Some new oxidation product may have been formed which acted as a fungicide. Exhaustive trials have demonstrated that dusts were equally as effective as sprays against blue mold. Those organic toxicants that were fungicidal as sprays, and were physically suitable for dusting, proved to be fungicidal also as dusts; *i.e.*, bismuth subsalicylate, Fermate, and zinc salicylate.

Certain combinations of fungicides gave a fungicidal effect greater than that obtained with single materials—that is, a synergistic effect. This is an important principle, and deserves further attention in disease-control practices. A synergistic combination may increase not only the fungicidal potency, but also allow the use of greatly reduced dosages. Organic fungicides appear to be particularly suitable for studies of this kind.

The residual protection provided by bismuth subsalicylate when applied as a spray or as a dust, and that of certain combined dusts containing zinc salicylate, is of both practical and theoretical interest. Its practical value is obvious. The writers saw no evidence that prolonged protection was due to more efficient coverage or better adhesion of these materials. Since adhesion was probably not a factor, it seems possible that a physiologic reaction between the salicylates and the plant tissues may have been involved. A similar phenomenon was observed by Clayton *et al.* (4), who pointed out that the protection provided by copper oxide–oil was not due to the same type of action usually found in sprays designed to give a protective layer of fungicide on exposed plant surfaces, and that such sprays as Bordeaux mixture were not effective against tobacco blue mold. Their suggestion was that the oil in the copper-oil spray acted in some indirect manner to promote resistance to blue mold.

SUMMARY

More than 250 organic toxicants and other materials were tested as blue-mold fungicides in the greenhouse and plant beds during 1941 through 1946 at Pee Dee Experiment Station, Florence, South Carolina. Extensive supplementary plant-bed tests were conducted also at Coastal Plain Experiment

Station, Tifton, Georgia, and the Tobacco Branch Experiment Stations at Oxford and McCullers, North Carolina. Bismuth subsalicylate was highly effective against blue mold and, in addition, benzyl salicylate, acetyl salicylate, salicylamide, salicylic acid, and sodium salicylate gave better control than copper oxide–oil. Certain derivatives of benzoic acid, especially benzoyl peroxide, were fungicidal. Fermate gave effective control in extensive experimental tests and in trials in commercial beds. In limited tests, Dithane D–14 gave satisfactory control but further testing is required for this material.

Certain organic fungicides controlled blue mold when used as dusts just as effectively as they had when used as sprays. Bismuth subsalicylate and Fermate both gave good results as dusts. Relatively large amounts of dust (17 to 20 lb. per 100 sq. yd. of bed area through the season) and thorough coverage were required. Combinations of zinc salicylate with Fermate, Thiosan, and Spergon were more effective than any of the components used alone.

Pronounced residual protection was secured by spraying or dusting with bismuth subsalicylate, or by dusting with combinations of zinc salicylate with Fermate and Thiosan.

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STABILITY OF LABILE VIRUSES IN DESICCATED TISSUE

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INTRODUCTION

In most climates the atmospheric moisture varies to such an extent that the drying of mosaic leaf tissue in laboratory atmosphere is subject to extreme irregularities, especially when whole leaves, attached to or detached from the stem, are used. Under humid conditions, whole leaves give up their moisture very slowly, fermentation processes destroy the plastid pigments, and the tissues do not become crisp. During periods of dry weather, such tissue dries more rapidly, but the plastid pigments are usually destroyed during the drying process, and, when dried under either of these conditions, many viruses do not survive for more than relatively short periods.

Working with the virus of cucumber mosaic, it was found that finely cut mosaic tobacco leaves dried in the laboratory sometimes contained traces of viable virus after the tissue had reached the stage commonly regarded as dry. However, no virus was recovered when whole leaves were similarly dried.

Since it is commonly known that fermentation processes are retarded at low temperatures, and that many of the labile viruses retain their activity for fairly long periods in expressed plant juices when held at or near freezing temperatures, it seemed reasonable that methods might be devised whereby certain of the labile viruses will retain a large part of their activity in dry tissue. A brief report of these studies with cucumber-mosaic virus has been published.[2]

METHODS AND MATERIALS

Leaf tissues were used throughout the studies. Desiccation was carried out in the free atmosphere and also in desiccators, made from baking pans $1\frac{1}{2} \times 7 \times 11$ inches. Calcium chloride crystals were placed in the bottom, a wire screen ($\frac{1}{2}$-inch mesh) above the crystals supported a layer of surgical gauze and the tissue. To prevent excessive absorption of tissue juices, the gauze was lightly sprayed with a solution of paraffin and any highly volatile solvent, and the solvent was allowed to evaporate. Care was exercised to avoid clogging the meshes of the gauze. Each pan was covered with a piece of glass cut to the dimensions of the pan. The edges of the glass were tightly taped to the sides of the pan with high grade zinc oxide tape 1 inch wide. This tape was better than cellulose acetate tape (Scotch tape), especially in making tight corners, and in the ease of removal.

Petri dishes of various sizes were very convenient for small samples. When these were used, the tops and the bottoms, respectively, were fitted in

[1] The author wishes to acknowledge the assistance of Matthew Koerner in connection with these studies.

[2] McKinney, H. H. Virus of cucumber mosaic withstands desiccation in leaf tissue. (Abstr.) Phytopath. **35**: 488. 1945.

pairs. Zinc oxide tape was used to cover the joint. Wire screen, surgical gauze, and tissue were placed over $CaCl_2$ crystals as in the pans.

Virus-infected leaves were removed from young, vigorous plants. With tobacco, the midribs were removed from large leaves and discarded. Tissues were clipped with shears into small pieces and spread on the surgical gauze in the pans or dishes.

After desiccation, the tissues were stored in bottles with screw caps. Caps were kept tightly in place to exclude moisture, or they were placed loosely on the bottles, and the bottles placed in tight cans containing $CaCl_2$ crystals. Storage was at 1°–2° C. unless noted otherwise.

Inoculations were made by wiping water extracts of the dried tissues on leaves dusted with carborundum powder.

All viruses were tested adequately at each assay to make certain that the surviving viruses were those under study, and not contaminating resistant viruses that were undetected in the original cultures.

The following viruses were studied:

Marmor cucumeris var. *vulgare* H.	Cucumber-mosaic virus
M. cucumeris var. *commolinae* H.	Southern celery-mosaic virus
M. anularium McK.[3]	Tobacco-ring-spot virus
M. cucumeris var. *upsilon* H.	Potato "Y"-mosaic virus
M. erodens H. (var. ?)	Tobacco-etch virus
M. medicaginis H.	Alfalfa-mosaic virus
M. terrestre var. *typicum* McK.[4]	Oat-apical-mosaic virus
M. terrestre var. *oculatum* McK.[4]	Oat-eyespot-mosaic virus
M. tritici var. *typicum* McK.[3]	Wheat-mosaic-rosette virus
M. tritici var. *fulvum* McK.[3]	Prairie-wheat-yellow-mosaic virus

These viruses become inactive in from a few days to a few weeks when the tissues are dried in the ordinary manner in the laboratory.

RESULTS

Since rapid drying reduces fermentation processes in leaf tissue, preliminary tests were conducted with desiccants at room temperature, and at higher temperatures with an isolate of cucumber-mosaic virus from squash and with the Southern celery-mosaic strain.

The results in table 1 show that some virus survived rapid desiccation of leaf tissues at the higher temperatures, but the level of survival seemed not sufficiently high to justify a full exploration of labile viruses by this method at this time.

The results in table 2 indicate that desiccation at temperatures just above freezing favors the survival of all but one of the labile viruses studied. No

[3] McKinney, H. H. Descriptions and revisions of several species of viruses in the genera Marmor, Fractilinia and Galla. Jour. Washington Acad. Sci. 34: 322–329. 1944.

[4] McKinney, H. H. Mosaics of winter oats induced by soil-borne viruses. Phytopath. 36: 359–369. 1946.

TABLE 1.—*Virus survival in leaf tissue desiccated at room temperature and above*

Virus	Host	Method of desiccating finely cut leaf tissue	Days dried	Test plant	Results[a]
Cucumber mosaic	Sweet corn	Dried in oven at 35° C. for 20 hr., stored over $CaCl_2$ at 23° C.	19	Tobacco	5/5
Do	do	do	39	do	2/5
Do	do	do	39	Corn	1/40
Do	do	do	58	Tobacco	2/2
Do	do	do	58	Corn	3/41
Do	do	Spread in lab. at 18.5° C.	9	do	0/45
Do	do	do	9	Tobacco	0/5
Do	do	In $CaCl_2$ desiccator at 18.5° C.	18	do	5/5
Do	do	do	42	do	2/2
Do	do	do	42	Corn	17/17
Do	Tobacco	Dried in oven at 53°–60° C. for 3¾ hr., tested immediately	.....	Tobacco	0/5
Southern celery mosaic	Sweet corn	Dried in oven at 35° C. for 22 hr., tested immediately	.....	Corn	1/32
Do	Cucumber	Dried in $CaCl_2$ desiccator in oven at 35° C. for 22 hr., tested immediately	.....	do	6/44
Do	Sweet corn	Dried in $CaCl_2$ desiccator in oven at 35° C. for 18 hr., stored at 1°–2° C.	27	do	2/112
Do	do	do	27	Cucumber	3/12
Do	do	$CaCl_2$ desiccator at 18.5° C.	24	do	1/13

[a] Numerators of fractions indicate the number of plants infected, and the denominators the number of plants inoculated.

tests were conducted at subfreezing temperatures, in oxygen-free atmosphere, or in vacuum to determine if further advantages may result.

DISCUSSION AND CONCLUSIONS

With the exception of the virus of oat eye-spot mosaic, the labile viruses tested survived desiccation for rather long periods. The oat-apical-mosaic virus showed slight activity after desiccation. However, as the oat-mosaic viruses are very difficult to transmit when the fresh extracts are used, the effect of desiccation is not apparent.

TABLE 2.—*Virus survival in finely cut leaf tissue desiccated over calcium chloride crystals at 1° to 2° C. Stored dry in tight bottles after desiccation at 1°–2° C.*

Virus	Host	Days dried	Test plant	Results[a]
Cucumber mosaic	Tobacco	125	Tobacco	5/5
Do	Sweet corn	669	do	5/5
Southern celery mosaic	do	613	do	5/5
Tobacco ring spot	Tobacco	393	do	5/5
Alfalfa mosaic	Cucumber	303	do	5/5
Potato "Y" mosaic	Tobacco	78	do	5/5
Do	do	420	do	6/10
Tobacco etch	Tobacco	301	do	10/10
Wheat mosaic rosette	Wheat	290	Wheat	10/38
Prairie wheat yellow mosaic	do	290	do	33/40
Oat apical mosaic	Oats	177	Oats	4/39
Oat eye-spot mosaic	do	177	do	0/41

[a] Numerators of fractions indicate the number of plants infected, and the denominators the number of plants inoculated.

Incidental tests have indicated that the host species is not a very important factor influencing survival of virus when desiccated. It appears that the major importance of the host lies in its ability to produce a large quantity of virus.

The results seem to justify the conclusion that, for the viruses studied, relatively rapid death in tissues dried in the ordinary ways at room temperature cannot be accounted for entirely on the basis of oxidation or desiccation. Desiccation may be a contributing factor, but it appears that other factors, such as fermentation processes, play a more direct rôle in bringing about this relatively rapid destruction of virus in tissues that are dried by the ordinary methods.

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PHYTOPATHOLOGICAL NOTES

Cuticle Cracks in Tomato Fruits.—Cracks in the cuticle of mature-sized green tomatoes (*Lycopersicon esculentum* Mill.) were first noticed at the Tomato Disease Laboratory at Jacksonville, Texas, on July 12, 1937 (Fig. 1). These cracks spoiled the appearance of the fruits but they appeared too late in the marketing season to cause serious economic loss. However, in the week of June 25 to July 1, 1945, farmers lost the No. 1 grade price on many carloads of green-wrap tomatoes due to cuticle cracking. This abnormality was closely correlated with the weather at that time. Rains totalling 1.38 inches fell on June 22 and 23. On June 24 the temperature was as

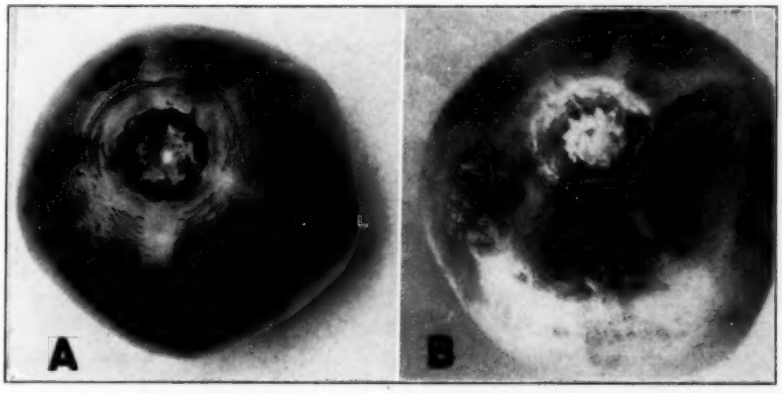

FIG. 1. A. Tomato fruit with cuticle cracks that became deep enough to expose the epidermal cells to air so that they became black. B. Tomato with areas of blackened and sunken cuticle cracks.

high as 90° F. and small cracks, visible only by reflected light, developed abundantly in the cuticle of most of the large green tomatoes in most fields. The cuticle cracks occurred only in the dark green tops within 2 cm. of the pedicels. The cracks became more abundant in the next two days when temperatures were 90° to 92° F. They remained hyaline during the first day or two until they deepened enough to cause drying of the epidermal cells when they became black or brown (Fig. 1, A). Within 5 days, the areas with numerous cuticle cracks became sunken and black, making spots 3 to 12 mm. in diameter (Fig. 1, B).

Similar cuticle cracks were less abundant in the green and red fruits of tomatoes in November, 1945. Most of these cracks were discernible only with reflected light because they remained hyaline. They had formed in the period between Nov. 11 and 21 at temperatures of 45° to 80° F. with only 0.1 inch of rain on five of these days. The soil contained adequate water from the 1.96 inches of rain on Nov. 9 and 10. The epidermis was stripped from some of the affected fruits, mounted in water on microscope slides, and examined to determine the nature of the striations. The cuticle cracks ranged from 25 to 3000 μ long, 12 to 45 μ wide, and 100 to 300 μ apart

(Fig. 2). The perpendicular fissures were about 24 μ long, 2 μ wide, and 3 to 6 μ apart in the longest crack illustrated (Fig. 2). A few of the cracks were branched.

Most of the cuticle cracks were short arcs of circles that were concentric around the pedicel and probably resulted from the spherical stretching of the cuticle that was associated with increase in the polar diameter of the fruit (Fig. 2, C, S). Some of these cracks apparently were stretched endwise soon after they formed (due to surface expansion that was associated with increase in the equatorial diameter), which made perpendicular fissures in some cracks (Fig. 2, F).

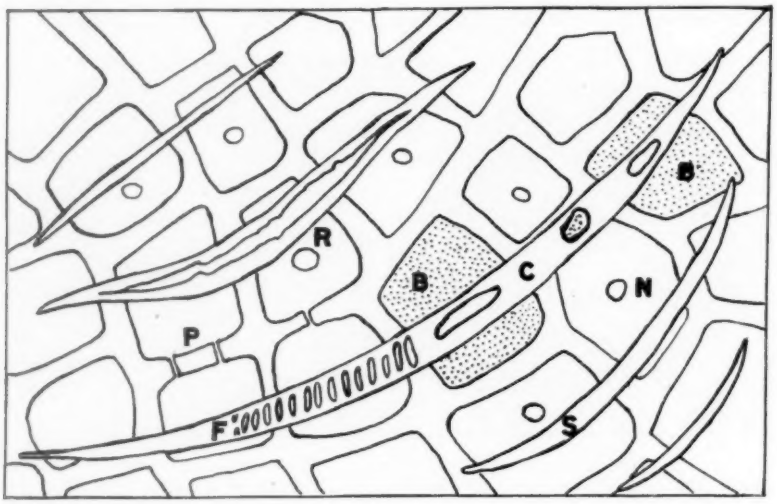

FIG. 2. Semidiagrammatic drawing of tomato-fruit epidermis with cuticle cracks above the epidermal cells. Shallow cuticle cracks (S) did not become deep enough to expose epidermal cells to air so they remained hyaline. Cuticle crack (C) deepened enough to expose two epidermal cells (B) to air so that they became black while three other underlying cells remained hyaline. Short fissures (F) formed almost perpendicular to the direction of the long cuticle crack that contained them. One cuticle crack (R) possibly ruptured first below the outer surface after which a ragged rift formed in the top. Nucleus (N). Plasmodesmata (P).

In 1946, abundant cuticle cracking was noticed first on June 24. It was associated with rains totaling 5.21 inches that occurred on June 20 and 21, and an additional 2.34 inches of rain fell in 9 showers by July 2, but the temperature remained in the range of 68° to 86° F. In recording yields on July 2 in a field of Rutgers tomatoes, 947 fruits were counted of which 18 per cent had cuticle cracking that was mild in about half of the fruits. Cuticle cracking was much more destructive in 1945 than in 1946, probably because hotter weather followed the rains in 1945.

The cuticle cracking described was in commercial varieties of green-wrap tomatoes with unripe fruits that had dark green stem-ends (due to UU-genes). Less cuticle cracking occurred in tomatoes that had uniformly colored whitish-green unripe fruits (due to the uu-genes). Four selections

of such tomatoes had only about one per cent of their fruits with cuticle cracking in 1946. Of these, Selection No. 3 of the Southern Tomato Exchange Program had one fruit with cuticle cracks that were bordered by brown epidermal cells. These cracks contained large air bubbles and small sand grains when the strip of tomato peel was mounted in water. Three of the selections of uu-gene tomatoes, including Crack-Proof variety, were resistant to both cuticle cracking and stem cracking.

Although they differ much in size and final symptoms, cuticle cracks probably have the same physiological causes as the deep subcuticular cracks that are commonly named stem cracks and growth cracks, and these two kinds of cracks often occurred in the same fruits.[1] The stem cracks were 1 to 5 mm. deep and 1 to 6 cm. long, and some cracks crossed the blossom-end of the fruits, especially in Trip-L-Crop variety. Rutgers and Marglobe tomatoes usually have radial stem cracks while Louisiana Red tomatoes usually have concentric stem cracks. This indicates that these dissimilar varieties differ in their resistance to cracking when the peel is stretched by the swelling that is associated with increase in equatorial diameter versus increase in polar diameter.

Groth[2] noticed cuticle cracks in tomato skin and mentioned that the colorless cells around the cracks were unusual because they did not stain with haematoxylin. Frazier[3] recorded a netting or russeting of Break O'Day tomatoes that may be related to cuticle cracking. Iverson[4] said that stem cracking resulted from application of water to the fruits or soil. Doolittle[5] illustrated common kinds of growth cracks. Most stem cracking was due to uneven periods of swelling of the fruits, and control depended on maintaining practically uniform water supply and rate of growth (Young[6]).—P. A. YOUNG, Texas Agricultural Experiment Station, Jacksonville, Texas.

Rusty Spot of Peach.—In 1941 the writer called attention to a peculiar condition affecting peach fruits in Idaho, the symptoms of which suggested the name rusty spot.[1] Subsequent observations indicate that the name is appropriate, particularly in the early stages of the disease (Fig. 1, C). The pubesence of portions of the young fruits eventually sloughs off, leaving unsightly, bald patches of varying sizes (Fig. 1, C, D). In comparison with normal-shaped pits those from severely affected rusty-spot fruits are smaller and thinner and the tips are curved toward the side of the severe rusty-spot area (Fig. 1, A, B).

[1] Young, P. A. Cuticle cracking in green tomato fruits. (Abstr.) Phytopath. **36**: 413. 1946.

[2] Groth, B. H. A. Structure of tomato skins. New Jersey Agr. Exp. Sta. Bul. 228. 1910.

[3] Frazier, W. A. Types and severity of fruit cracking in tomato varieties. Proc. Amer. Soc. Hort. Sci. **34**: 536. 1937.

[4] Iverson, V. E. Fruit cracking of tomatoes. Montana Agr. Exp. Sta. Bul. 362. 1938.

[5] Doolittle, S. P. Tomato diseases. U. S. Dept. Agr. Farmers Bul. 1934. 1943.

[6] Young, R. E. Trellis tomatoes. Massachusetts Agr. Exp. Sta. Bul. 419. 1944.

[1] Blodgett, Earle C. Rusty spot of peach. U. S. Dept. Agr., Pl. Dis. Rptr. **25**: 27–28. 1941.

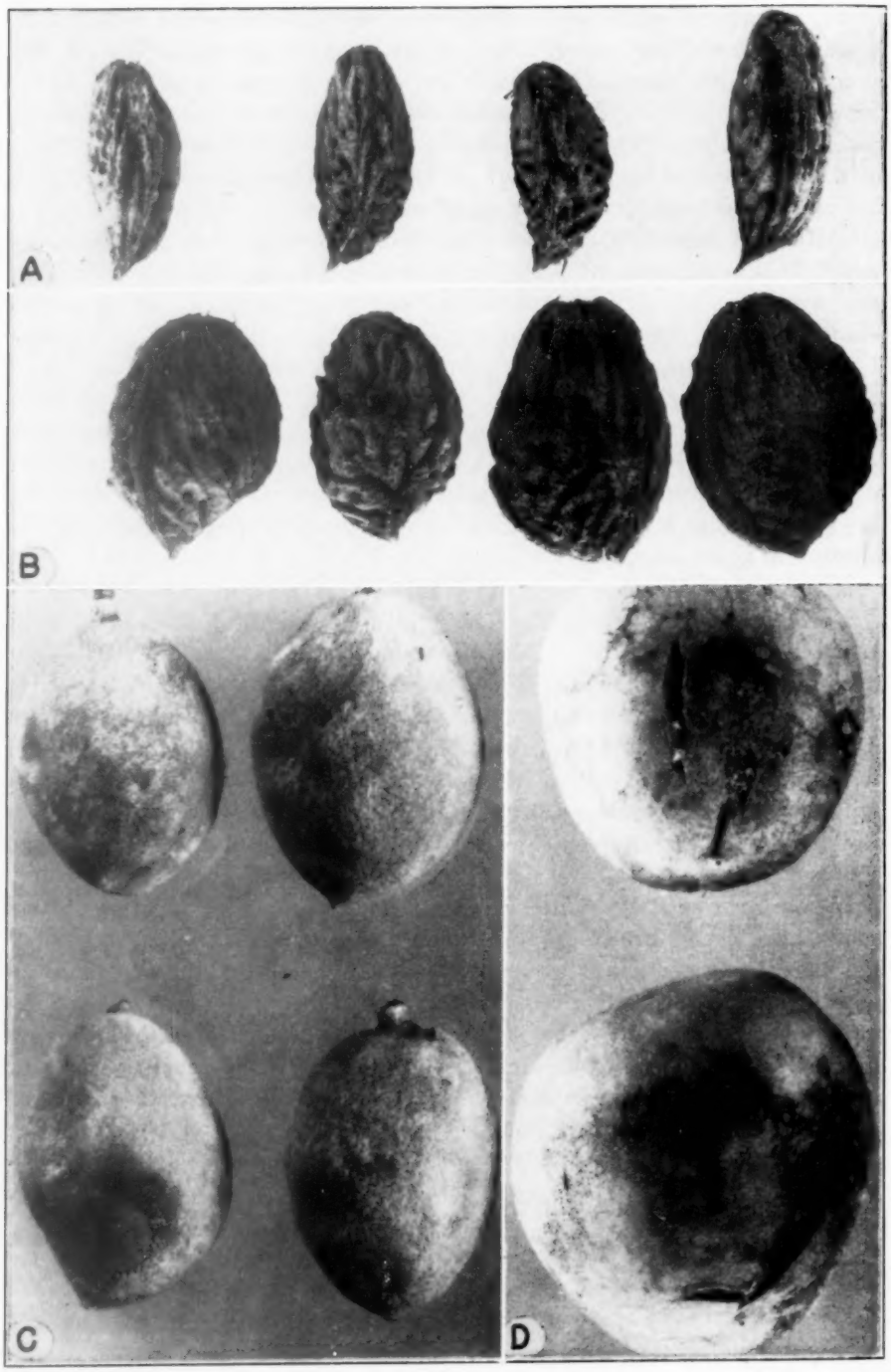

FIG. 1. Rusty spot of Elberta peach naturally affected. A. Seeds of severely affected rusty-spot fruits showing the small, curved pits. B. Normal peach seeds. C. Young fruits showing the very early stages of rusty spot. D. Nearly mature fruits showing severely affected areas resulting in russeting, cracking, and malformation.

Although the exact nature of the cause of rusty spot is not yet determined, renewed interest is shown over this peculiar disease. In personal correspondence with E. L. Reeves in Washington, Gilbert Stout in California, and H. R. McLarty in British Columbia, the presence of rusty spot in these areas has been noted. In a few cases in California and Washington the disease has assumed serious proportions, especially on certain varieties.

In September, 1940, in the plots at Moscow, Idaho, 7 peach seedlings were bud-inoculated with 3 buds each of material from the orchard where the disease was first reported. No tree or leaf symptoms were noted until August, 1945, when it was observed that most of the fruit on 6 of the bud-inoculated trees were slightly to severely affected with rusty spot. One bud-inoculated tree bore healthy but no rusty-spot fruit.

Five of the bud-inoculated trees produced typically affected fruit; one tree bearing 21 rusty-spot and 3 healthy fruit, and another tree bearing 22 rusty-spot and 11 healthy fruit. Unfortunately, it was impossible to ascertain with certainty whether these 5 trees represented the under stock or the development of the scion. Fruit type was typical of the Elberta grown in the original orchard and the trees probably are from the scion shoots.

One of the bud-inoculated trees, however, which bore affected fruit was undoubtedly a seedling and represented the original under stock. The fruit was round and green while that from the other trees was elongated and more nearly mature. There is therefore indication that, in this case, the factor causing rusty spot was either transmitted or provided contamination from the scion buds. Adjacent trees and other trees in the plots, some checks and some inoculated with various bud-wood collections, showed no similarly affected fruit except in one case of an adjacent tree. This was a peach seedling which was inoculated in 1940 with buds from red-leaf chokecherry and bore only one rusty spot and several healthy peach fruits. Further observation substantiates that the rusty spots on the fruits are not due to infection by the ordinary powdery-mildew fungus.

Whether the factor is transmissible (as a virus), or perpetuated (as a genetic abnormality), or carried mechanically (as an organism), the results point out the danger of using bud wood from affected trees for propagation purposes. The occurrence of rusty spot on one tree not inoculated with affected material indicates the possibility of orchard spread.—EARLE C. BLODGETT, Formerly at Idaho Agricultural Experiment Station, Moscow, Idaho.